Susan King Strasinger

Marjorie Schaub Di Lorenzo

Urinalysis and Body Fluids

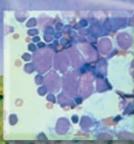
FIFTH EDITION

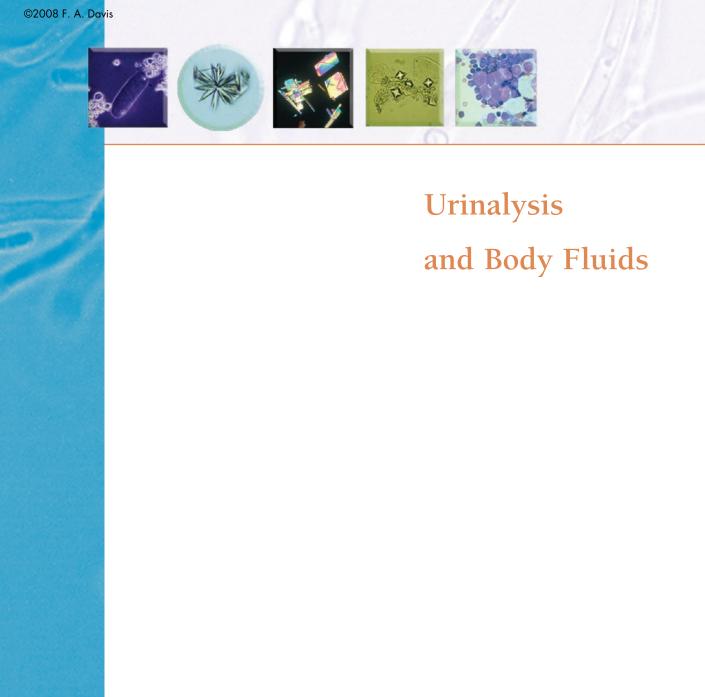






















Urinalysis and Body Fluids

Fifth Edition

Susan King Strasinger, DA, MT(ASCP)

Faculty Associate The University of West Florida Pensacola, Florida

Marjorie Schaub Di Lorenzo, BS, MT(ASCP)SH

Adjunct Instructor
Division of Laboratory Sciences
Clinical Laboratory Science Program
University of Nebraska Medical Center
Omaha, Nebraska
Phlebotomy Program Coordinator
Health Professions
Nebraska Methodist College
Omaha, Nebraska

F. A. Davis Company 1915 Arch Street Philadelphia, PA 19103 www.fadavis.com

Copyright © 2008 by F. A. Davis Company

Copyright © 2008 by F. A. Davis Company. All rights reserved. This product is protected by copyright. No part of it may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without written permission from the publisher.

Printed in the United States of America

Last digit indicates print number: 10 9 8 7 6 5 4 3 2 1

Acquisitions Editor: Christa Fratantoro Manager of Content Development: Deborah Thorp Manager of Art and Design: Carolyn O'Brien

As new scientific information becomes available through basic and clinical research, recommended treatments and drug therapies undergo changes. The author(s) and publisher have done everything possible to make this book accurate, up to date, and in accord with accepted standards at the time of publication. The author(s), editors, and publisher are not responsible for errors or omissions or for consequences from application of the book, and make no warranty, expressed or implied, in regard to the contents of the book. Any practice described in this book should be applied by the reader in accordance with professional standards of care used in regard to the unique circumstances that may apply in each situation. The reader is advised always to check product information (package inserts) for changes and new information regarding dose and contraindications before administering any drug. Caution is especially urged when using new or infrequently ordered drugs.

Library of Congress Cataloging-in-Publication Data

Strasinger, Susan King.

Urinalysis and body fluids / Susan King Strasinger, Marjorie Schaub Di Lorenzo; photography by Bo Wang ... [et al.]; illustrations by Sherman Bonomelli. — 5th ed.

p. ; cm

includes bibliographical references and index.

ISBN 978-0-8036-1697-4 (alk. paper)

1. Urine—Analysis. 2. Body fluids—Analysis. 3. Diagnosis, Laboratory. I. Di Lorenzo, Marjorie Schaub, 1953- II. Title.

[DNLM: 1. Urinalysis—methods. 2. Body Fluids—chemistry. QY 185 S897u 2008] RB53.S87 2008

616.07'566—dc22 2007017271

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by F. A. Davis Company for users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that the fee of \$.10 per copy is paid directly to CCC, 222 Rosewood Drive, Danvers, MA 01923. For those organizations that have been granted a photocopy license by CCC, a separate system of payment has been arranged. The fee code for users of the Transactional Reporting Service is: \$036-1698/08 + \$.10.

©2008 F. A. Davis

To Harry, you will always be my Editor-in-Chief SKS

To my husband, Scott, and my children, Michael, Christopher, and Lauren



Preface

As will be apparent to the readers, the fifth edition of *Urinal-ysis and Body Fluids* has been substantially revised and enhanced. However, the objective of the text—to provide concise, comprehensive, and carefully structured instruction in the analysis of nonblood body fluids—remains the same.

This fifth edition has been redesigned to meet the changes occurring in both laboratory medicine and instructional methodology.

To meet the expanding technical information required by students in laboratory medicine, all of the chapters have been updated. Chapter 1 is devoted to overall laboratory safety and the precautions relating to urine and body fluid analysis. Chapter 7 addresses quality assessment and management in the urinalysis laboratory. Preanalytical, analytical, and postanalytical factors, procedure manuals, current regulatory issues, and methods for continuous quality improvement are stressed. In Chapter 8 the most frequently encountered diseases of glomerular, tubular, interstitial, and vascular origin are related to their associated laboratory tests. To accommodate advances in laboratory testing of cerebrospinal, seminal, synovial, serous, and amniotic fluids, all of the individual chapters have been enhanced, and additional anatomy and physiology sections have been added for each of these fluids. Appendix A provides coverage of the ever-increasing variety of automated instrumentation available to the urinalysis laboratory. Appendix B discusses the

analysis of bronchioalveolar lavage specimens, an area of the clinical laboratory that has been expanding in recent years.

This fifth edition has been redesigned to include extensive multiple choice questions at the end of each chapter for student review. In response to readers' suggestions, the number of color slides has been significantly increased, and the slides are included within the text to increase user friendliness. The text has been extensively supplemented with tables, summaries, and procedure boxes, and many figures are now in full color. Case studies in the traditional format and clinical situations relating to technical considerations are included at the end of the chapters. Answers to the study questions, case studies, and clinical situations are also included at the end of the book. Terms in bold italics appear in the Glossary; abbreviations in **bold** are listed in Abbreviations. Additional support is provided to adopting instructors in the form of accompanying test-generating software, an instructor's manual with criticial thinking exercises for each chapter, and PowerPoint presentations.

We have given consideration to the suggestions of our previous readers and believe these valuable suggestions have enabled us to produce a text to meet the needs of all users.

Susan King Strasinger

Marjorie Schaub Di Lorenzo



Reviewers

Ellen P. Digan, MA, MT(ASCP)

Professor of Biology Coordinator of Medical Laboratory Technology Program Manchester Community Tech College Department of Math, Science, and Health Careers Manchester, Connecticut

Brenda L. M. Franks, MT(ASCP)

Point of Care Testing Coordinator Methodist Hospital Pathology Omaha, Nebraska

Stephen M. Johnson, MS, MT(ASCP)

Program Director Medical Technology Saint Vincent Health Center Erie, Pennsylvania

Rhoda S. Jost, MSH, MT(ASCP)

Faculty Program Director Medical Laboratory Technology Florida Community College at Jacksonville Jacksonville, Florida

Pam Kieffer, MS, CLS(MCA), MT(ASCP)

Program Director Clinical Laboratory Science Rapid City Regional Hospital Rapid City, South Dakota

Cynthia A. Martine, MEd, MT(ASCP)

Assistant Professor Department of Clinical Laboratory Sciences University of Texas Medical Branch School of Allied Health Galveston, Texas

Ulrike Otten, MT(ASCP)SC

University of Nebraska Medical Center Division of Laboratory Sciences Clinical Laboratory Science Program Omaha, Nebraska

Kathleen T. Paff, MA, CLS(NCA), MT(ASCP)

Program Director Medical Laboratory Technology Kellogg Community College Battle Creek, Michigan

Kristy Shanahan, MS, NCA, MT(ASCP)

Associate Professor Medical Laboratory Technology Oakton Community College Des Plaines, Illinois

Amber G.Tuten, MEd, CLDir(NCA), MT(ASCP)

Program Director Medical Laboratory Technology Okefenokee Technical College Waycross, Georgia



Acknowledgments

Many people deserve credit for the help and encouragement they have provided us in the preparation of this fifth edition. Our continued appreciation is also extended to all of the people who have been instrumental in the preparation of previous editions.

The valuable suggestions from previous readers and the support from our colleagues at The University of West Florida, Northern Virginia Community College, The University of Nebraska Medical Center, Methodist Hospital, and Creighton University Medical Center have been a great asset to us in the production of this new edition. We thank each and every one of you.

We extend special thanks to the individuals who have provided us with so many beautiful photographs for the text over the years: Bo Wang, MD; Donna L. Canterbury, BA, MT(ASCP)SH; Joanne M. Davis, BS, MT(ASCP)SH; M. Paula Neumann, MD; Gregory J. Swedo, MD; and Scott Di Lorenzo, DDS. We also thank Sherman Bonomelli, MS, for contributing original visual concepts that became the foundation for many of the line illustrations.

We also appreciate the help and understanding of our editors at F. A. Davis, Christa Fratantoro, Elizabeth Zygarewicz, and Deborah Thorp.



Contents

Chapter I. Safety in the Clinical Laboratory, I

Biological Hazards, 2

Personal Protective Equipment, 4

Handwashing, 4

Disposal of Biological Waste, 5

Sharp Hazards, 5

Chemical Hazards, 6

Chemical Spills, 6

Chemical Handling, 6

Chemical Hygiene Plan, 6

Chemical Labeling, 6

Material Data Safety Sheets, 7

Radioactive Hazards, 7

Electrical Hazards, 7

Fire/Explosive Hazards, 8

Physical Hazards, 8

Chapter 2. Renal Function, II

Renal Physiology, 12

Renal Blood Flow, 12

Glomerular Filtration, 13

Tubular Reabsorption, 14

Tubular Secretion, 17

Renal Function Tests, 18

Glomerular Filtration Tests, 18

Tubular Reabsorption Tests, 22

Tubular Secretion and

Renal Blood Flow Tests, 24

Chapter 3. Introduction to Urinalysis, 29

History and Importance, 29

Urine Formation, 31

Urine Composition, 31

Urine Volume, 31

Specimen Collection, 32

Specimen Handling, 33

Specimen Integrity, 33

Specimen Preservation, 33

Types of Specimens, 34

Random Specimen, 34

First Morning Specimen, 34

Fasting Specimen (Second Morning), 34

2-Hour Postprandial Specimen, 35

Glucose Tolerance Specimens, 35

24-Hour (or Timed) Specimen, 36

Catheterized Specimen, 36

Midstream Clean-Catch Specimen, 36

Suprapubic Aspiration, 36

Prostatitis Specimen, 36

Pediatric Specimen, 37

Drug Specimen Collection, 37

Chapter 4. Physical Examination of Urine, 41

Color, 42

Normal Urine Color, 42

Abnormal Urine Color, 43

Clarity, 44

Normal Clarity, 44

Nonpathologic Turbidity, 44

Pathologic Turbidity, 45

Specific Gravity, 45

Urinometer, 46

Refractometer, 47

Harmonic Oscillation Densitometry, 48

Clinical Correlations, 48

Odor, 49

xiv Contents

Clinical Significance, 68

Chapter 5. Chemical Examination of Urine, 53	Reagent Strip (Diazo) Reactions, 68 Ictotest Tablets, 68		
Reagent Strips, 54	Reaction Interference, 69		
Reagent Strip Technique, 54	Urobilinogen, 69		
Handling and Storage of Reagent Strips, 55	Clinical Significance, 70		
Quality Control of Reagent Strips, 55	Reagent Strip Reactions and Interference, 70		
pH, 56	Reaction Interference, 70		
Clinical Significance, 56	Ehrlich Tube Test, 70		
Reagent Strip Reactions, 56	Watson-Schwartz Differentiation Test, 71		
Protein, 57	Hoesch Screening Test		
Clinical Significance, 57	for Porphobilinogen, 71		
Prerenal Proteinuria, 57	Nitrite, 72		
Renal Proteinuria, 58	Clinical Significance, 72		
Postrenal Proteinuria, 58	Reagent Strip Reactions, 72		
Reagent Strip Reactions, 58	Reaction Interference, 73		
Reaction Interference, 59	Leukocyte Esterase, 73		
Glucose, 6 l	Clinical Significance, 73		
Clinical Significance, 62	Reagent Strip Reaction, 74		
Reagent Strip (Glucose	Reaction Interference, 74		
Oxidase) Reactions, 62	Specific Gravity, 74		
Reaction Interference, 63	Reagent Strip Reaction, 75		
Copper Reduction Test, 63	Reaction Interference, 75 Chapter 6. Microscopic Examination of Urine, 81		
Comparison of Glucose Oxidase and Clinitest, 64			
Ketones, 64			
Clinical Significance, 64	Macroscopic Screening, 82		
Reagent Strip Reactions, 65	Preparation and Examination		
Reaction Interference, 65	of the Urine Sediment, 82		
Blood, 65	Commercial Systems, 82		
Clinical Significance, 66	Specimen Preparation, 83		
Hematuria, 66	Specimen Volume, 83		
Hemoglobinuria, 66	Centrifugation, 83		
Myoglobinuria, 66	Sediment Preparation, 83		
Hemoglobinuria Versus Myoglobinuria, 67	Volume of Sediment Examined, 83		
Reagent Strip Reactions, 67	Examination of the Sediment, 83		
Reaction Interference, 67	Reporting the Microscopic Examination, 84		
Bilirubin, 68	Correlation of Results, 84		
Production of Bilirubin, 68	Sediment Examination Techniques, 84		

Sediment Stains, 85

Cytodiagnostic Urine Testing, 87	Minimal Change Disease, 146		
Microscopy, 87	•		
Types of Microscopy, 89	Focal Segmental Glomerulosclerosis, 146 Alport Syndrome, 147		
	• •		
Sediment Constituents, 92	Diabetic Nephropathy, 147		
Red Blood Cells, 92	Tubular Disorders, 147		
White Blood Cells, 94	Acute Tubular Necrosis, 149		
Epithelial Cells, 95	Hereditary and Metabolic Tubular Disorders, 149		
Bacteria, 100	Fanconi Syndrome, 149		
Yeast, 100	Nephrogenic Diabetes Insipidus, 149		
Parasites, 100	Renal Glycosuria, 149		
Spermatozoa, 100	Interstitial Disorders, 149		
Mucus, 102	Acute Pyelonephritis, 150		
Casts, 102	Chronic Pyelonephritis, I50		
Urinary Crystals, 110	Acute Interstitial Nephritis, 151		
Urinary Sediment Artifacts, 119	Renal Failure, 151		
Chapter 7. Quality Assessment and Management in the Urinalysis	Renal Lithiasis, 152		
Laboratory, 127 Urinalysis Procedure Manual, 128	Chapter 9. Urine Screening for Metabolic Disorders, 159		
Preanalytical Factors, 129	Overflow Versus		
Analytical Factors, 129	Renal Disorders, 160		
Postanalytical Factors, 134	Newborn Screening Tests, 160		
Regulatory Issues, 135	Amino Acid Disorders, 161 Phenylalanine-Tyrosine Disorders, 161		
Quality Management, 137			
Medical Errors, 139	Branched-Chain Amino Acid Disorders, 164		
	Tryptophan Disorders, 165		
Chapter 8. Renal Disease, 143	Cystine Disorders, 166		
Glomerular Disorders, 144	Porphyrin Disorders, 167		
Glomerulonephritis, 144	Historical Note, 168		
Acute Poststreptococcal Glomerulonephritis, 144	Mucopolysaccharide Disorders, 169		
Rapidly Progressive (Crescentic)	Purine Disorders, 170		
Glomerulonephritis, 144	Carbohydrate Disorders, I70		
Goodpasture Syndrome, 144			
Membranous Glomerulonephritis, 145	Chapter 10. Cerebrospinal Fluid, 177		
Membranoproliferative Glomerulonephritis, I 45	Formation and Physiology, 178 Specimen Collection and Handling, 178		
Chronic Glomerulonephritis, 145			
Immunogloblin A Nephropathy, 145	Appearance, I79		
Nephrotic Syndrome, I45	Traumatic Collection (Tap), 179		

xvi

CONTENTS

Uneven Distribution of Blood, 179	Additional Testing, 205		
Clot Formation, 180	Sperm Viability, 205		
Xanthochromic Supernatant, 180	Seminal Fluid Fructose, 206		
Cell Count, 180	Antisperm Antibodies, 206		
Methodology, 181	Microbial and Chemical Testing, 207		
Total Cell Count, 181	Postvasectomy Semen Analysis, 207		
WBC Count, 181	Sperm Function Tests, 207		
Corrections for Contamination, 182	Semen Analysis Quality Control, 207		
Quality Control of Cerebrospinal Fluid and Other Body Fluid Cell Counts, 182	Chapter 12. Synovial Fluid, 211		
Differential Count on a Cerebrospinal Fluid Specimen, 182	Physiology, 211		
Cytocentrifugation, 182	Specimen Collection and Handling, 212		
Cerebrospinal Fluid Cellular Constituents, 183	Color and Clarity, 213		
Chemistry Tests, 189	Viscosity, 213		
Cerebrospinal Protein, 189	Cell Counts, 213		
Cerebrospinal Fluid Glucose, 191	Differential Count, 213		
Cerebrospinal Fluid Lactate, 192	Crystal Identification, 214		
Cerebrospinal Fluid Glutamine, 192	Types of Crystals, 214		
•	Slide Preparation, 215		
Microbiology Tests, 192	Crystal Polarization, 216		
Gram Stain, 193	Chemistry Tests, 217		
Serologic Testing, 194	Microbiologic Tests, 217		
Teaching Cerebrospinal Fluid Analysis, 195	Serologic Tests, 218		
Chapter II. Semen, 199	Chapter 13. Serous Fluid, 221		
Physiology, 199	Formation, 22 I		
Specimen Collection, 200	Specimen Collection and Handling, 222		
Specimen Handling, 201	Transudates and Exudates, 223		
Semen Analysis, 201	General Laboratory Procedures, 223		
Appearance, 201	Pleural Fluid, 223		
Liquefaction, 201	Appearance, 224		
Volume, 201	Hematology Tests, 225		
Viscosity, 202	Chemistry Tests, 226		
рН, 202	Microbiologic and Serologic Tests, 227		
Sperm Concentration/Count, 202	Pericardial Fluid, 228		
Sperm Motility, 203	Appearance, 228		
Sperm Morphology, 203	Laboratory Tests, 228		

Foam Stability, 240

Density, 241

Microviscosity: Fluorescence Polarization Assay, 241

Lamellar Bodies and Optical

Peritoneal Fluid, 229 Chapter 15. Fecal Analysis, 245 Transudates Versus Exudates, 229 Physiology, 245 Appearance, 230 Diarrhea, 246 Laboratory Tests, 230 Steatorrhea, 248 Specimen Collection, 248 Chapter 14. Amniotic Fluid, 235 Macroscopic Screening, 248 Physiology, 235 Color, 248 Function, 235 Appearance, 248 Volume, 236 Microscopic Examination Chemical Composition, 236 of Feces, 249 **Differentiating Maternal Urine** Fecal Leukocytes, 249 From Amniotic Fluid, 237 Muscle Fibers, 249 **Specimen Collection, 237 Qualitative Fecal Fats, 249** Indications for Amniocentesis, 237 Chemical Testing of Feces, 250 **Indications for Performing Amniocentesis, 237** Occult Blood, 250 Collection, 237 Quantitative Fecal Fat Testing, 25 I Specimen Handling APT Test (Fetal Hemoglobin), 252 and Processing, 237 Fecal Enzymes, 253 Color and Appearance, 238 Carbohydrates, 253 Tests for Fetal Distress, 238 Hemolytic Disease of the Newborn, 238 Appendix A, 259 **Neural Tube Defects, 239** Appendix B, 265 Tests for Fetal Maturity, 239 **Answers to Case Studies** Fetal Lung Maturity, 239 and Clinical Situations, 267 Lecithin-Sphingomyelin Ratio, 240 **Answers to Study Questions, 273** Amniostat-FLM, 240

Abbreviations, 277

Glossary, 279

Index, 285











Safety in the Clinical Laboratory

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

- 1 List the components of the chain of infection and the laboratory safety precautions that break the chain.
- **2** Differentiate among and state the precautions addressed by Universal Precautions, body substance isolation, and Standard Precautions.
- **3** State the specifics of the Occupational Exposure to Blood-Borne Pathogens Standard.
- 4 Describe the types of personal protective equipment that laboratory personnel wear, including when, how, and why each article is used.
- **5** Correctly perform routine handwashing.
- **6** Describe the acceptable methods for disposing of biological waste and sharp objects in the urinalysis laboratory.

- 7 Discuss the components and purpose of chemical hygiene plans and material safety data sheets.
- **8** State the components of the National Fire Protection Association hazardous material labeling system.
- 9 Describe precautions that laboratory personnel should take with regard to radioactive and electrical hazards.
- **10** Explain the RACE and PASS actions to be taken when a fire is discovered.
- 11 Differentiate among class A, B, C, and D fires with regard to material involved and methods of extinguishing each type.
- 12 Recognize standard hazard warning symbols.

KEY TERMS

biohazardous chain of infection chemical hygiene plan Material Safety Data Sheet Occupational Safety and Health Administration (OSHA) personal protective equipment (PPE) postexposure prophylaxis
(PEP)
radioisotope
Standard Precautions
Universal Precautions (UP)

2 CHAPTER I • Safety in the Clinical Laboratory

Table 1–1	Types of Safety Hazards	
Туре	Source	Possible Injury
Biological	Infectious agents	Bacterial, fungal, viral, or parasitic infections
Sharps	Needles, lancets, broken glass	Cuts, punctures, or blood-borne pathogen exposure
Chemical	Preservatives and reagents	Exposure to toxic, carcinogenic, or caustic agents
Radioactive	Equipment and radioisotopes	Radiation exposure
Electrical	Ungrounded or wet equipment; frayed cords	Burns or shock
Fire/explosive	Bunsen burners, organic chemicals	Burns or dismemberment
Physical	Wet floors, heavy boxes, patients	Falls, sprains, or strains
From Strasinger, SK and DiLorenzo, MA: Phlebotomy Workbook for the Multiskilled Healthcare Professional, FA Davis, Philadelphia,		

The clinical laboratory contains a variety of safety hazards, many of which are capable of producing serious injury or life-threatening disease. To work safely in this environment, laboratory personnel must learn what hazards exist, the basic safety precautions associated with them, and how to apply the basic rules of common sense required for everyday safety. As can be seen in Table 1–1, some hazards are unique to the health-care environment, and others are encountered routinely throughout life.

■■● Biological Hazards

1996, p 62, with permission.



The health-care setting provides abundant sources of potentially harmful microorganisms. These microorganisms are frequently present in the specimens received in the clinical laboratory. Understanding

how microorganisms are transmitted (*chain of infection*) is essential to preventing infection. The chain of infection requires a continuous link between a source, a method of transmission, and a susceptible host. The source is the location of potentially harmful microorganisms, such as a contaminated clinical specimen or an infected patient. Microorganisms

from the source are transmitted to the host. This may occur by direct contact (e.g., the host touches the patient, specimen, or a contaminated object), inhalation of infected material (e.g., aerosol droplets from a patient or an uncapped centrifuge tube), ingestion of a contaminated substance (e.g., food, water, specimen), or from an animal or insect vector bite. Once the chain of infection is complete, the infected host then becomes another source able to transmit the microorganisms to others.

In the clinical laboratory, the most direct contact with a source of infection is through contact with patient specimens, although contact with patients and infected objects also occurs. Preventing completion of the chain of infection is a primary objective of biological safety. Figure 1-1 uses the universal symbol for *biohazardous* material to demonstrate how following prescribed safety practices can break the chain of infection. Figure 1-1 places particular emphasis on laboratory practices.

Proper handwashing and wearing *personal protective equipment* (PPE) are of major importance in the laboratory. Concern over exposure to blood-borne pathogens, primarily hepatitis B virus (HBV) and human immunodeficiency virus (HIV), resulted in the drafting of guidelines and regulations

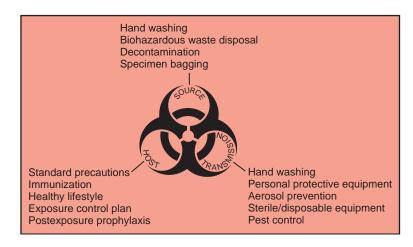


Figure I–I Chain of infection and safety practices related to the biohazard symbol. (Adapted from Strasinger, SK and DiLorenzo, MA: Phlebotomy Workbook for the Multiskilled Healthcare Professional, FA Davis, Philadelphia, 1996, p 62, with permission.)

by the Centers for Disease Control and Prevention (CDC) and the Occupational Safety and Health Administration (OSHA) to prevent exposure. In 1987 the CDC instituted Universal Precautions (UP). Under UP all patients are considered to be possible carriers of blood-borne pathogens. The guideline recommends wearing gloves when collecting or handling blood and body fluids contaminated with blood and wearing face shields when there is danger of blood splashing on mucous membranes and when disposing of all needles and sharp objects in puncture-resistant containers. The CDC excluded urine and body fluids not visibly contaminated by blood from UP, although many specimens can contain a considerable amount of blood before it becomes visible. The modification of UP for body substance isolation (BSI) helped to alleviate this concern. BSI guidelines are not limited to blood-borne pathogens; they consider all body fluids and moist body substances to be potentially infectious. According to BSI guidelines, personnel should wear gloves at all times when encountering moist body substances. A major disadvantage of BSI guidelines are that they do not recommend handwashing following removal of gloves unless visual contamination is present.

In 1996 the CDC combined the major features of UP and BSI guidelines and called the new guidelines *Standard Precautions*. Although Standard Precautions, as described below, stress patient contact, the principles most certainly can also be applied to handling patient specimens in the laboratory. Standard Precautions are as follows:

- 1. Handwashing: Wash hands after touching blood, body fluids, secretions, excretions, and contaminated items, whether or not gloves are worn. Wash hands immediately after gloves are removed, between patient contacts, and when otherwise indicated to avoid transfer of microorganisms to other patients or environments. Washing hands may be necessary between tasks and procedures on the same patient to prevent cross-contamination of different body sites.
- 2. Gloves: Wear gloves (clean, nonsterile gloves are adequate) when touching blood, body fluids, secretions, excretions, and contaminated items. Put on gloves just before touching mucous membranes and nonintact skin. Change gloves between tasks and procedures on the same patient after contact with material that may contain a high concentration of microorganisms. Remove gloves promptly after use, before touching noncontaminated items and environmental surfaces, and before going to another patient. Always wash your hands immediately after glove removal to avoid transfer of microorganisms to other patients or environments.
- 3. Mask, eye protection, and face shield: Wear a mask and eye protection or a face shield to protect mucous membranes of the eyes, nose, and mouth during procedures and patient care activities that are likely to generate splashes or sprays of blood, body fluids,

- secretions, or excretions. A specially fitted respirator (N95) must be used during patient care activites related to suspected mycobacterium exposure.
- 4. **Gown:** Wear a gown (a clean, nonsterile gown is adequate) to protect skin and to prevent soiling of clothing during procedures and patient care activities that are likely to generate splashes or sprays of blood, body fluids, secretions, or excretions. Select a gown that is appropriate for the activity and the amount of fluid likely to be encountered (e.g., fluid-resistant in the laboratory). Remove a soiled gown as promptly as possible, and wash hands to avoid the transfer of microorganisms to other patients or environments.
- 5. Patient care equipment: Handle used patient care equipment soiled with blood, body fluids, secretions, and excretions in a manner that prevents skin and mucous membrane exposures, contamination of clothing, and transfer of microorganisms to other patients or environments. Ensure that reusable equipment is not used for the care of another patient until it has been cleaned and reprocessed appropriately. Ensure that single-use items are discarded properly.
- 6. **Environmental control:** Ensure that the hospital has adequate procedures for the routine care, cleaning, and disinfection of environmental surfaces, beds, bedrails, bedside equipment, and other frequently touched surfaces. Ensure that these procedures are being followed.
- 7. Linen: Handle, transport, and process linen soiled with blood, body fluids, secretions, and excretions in a manner that prevents skin and mucous membrane exposures and contamination of clothing and that avoids the transfer of microorganisms to other patients and environments.
- 8. Occupational health and blood-borne pathogens: Take care to prevent injuries when using needles, scalpels, and other sharp instruments or devices; when handling sharp instruments after procedures; when cleaning used instruments; and when disposing of used needles. Never recap used needles or otherwise manipulate them using both hands or use any other technique that involves directing the point of a needle toward any part of the body; rather, use self-sheathing needles or a mechanical device to conceal the needle. Do not remove used needles from disposable syringes by hand, and do not bend, break, or otherwise manipulate used needles by hand. Place used disposable syringes and needles, scalpel blades, and other sharp items in appropriate puncture-resistant containers, which are located as close as practical to the area in which the items were used, and place reusable syringes and needles in a puncture-resistant container for transport to the reprocessing area. Use mouthpieces, resuscitation bags, or other ventilation devices as an alternative

4 CHAPTER I • Safety in the Clinical Laboratory

to mouth-to-mouth resuscitation methods in areas where the need for resuscitation is predictable.

9. Patient placement: Place a patient who contaminates the environment or who does not (or cannot be expected to) assist in maintaining appropriate hygiene or environment control in a private room. If a private room is not available, consult with infection control professionals regarding patient placement or other alternatives.

The Occupational Exposure to Blood-Borne Pathogens Standard is a law monitored and enforced by OSHA.² Specific requirements of this OSHA standard include the following:

- 1. Requiring all employees to practice UP/Standard Precautions
- 2. Providing laboratory coats, gowns, face and respiratory protection, and gloves to employees and laundry facilities for nondisposable protective clothing
- 3. Providing sharps disposal containers and prohibiting recapping of needles
- 4. Prohibiting eating, drinking, smoking, and applying cosmetics, lip balm, and contact lens in the work area
- 5. Labeling all biohazardous material and containers
- 6. Providing free immunization for HBV
- 7. Establishing a daily disinfection protocol for work surfaces; an appropriate *disinfectant* for blood-borne pathogens is sodium hypochlorite (household bleach diluted 1:10)
- 8. Providing medical follow-up for employees who have been accidentally exposed to blood-borne pathogens
- 9. Documenting regular training in safety standards for employees

Any accidental exposure to a possible blood-borne pathogen must be immediately reported. Evaluation of the incident must begin right away to ensure appropriate *postex-posure prophylaxis* (PEP). The CDC provides periodically updated guidelines for the management of exposures and recommended PEP.^{3,4}

Personal Protective Equipment

PPE used in the laboratory includes gloves, fluid-resistant gowns, eye and face shields, and Plexiglas countertop shields. Gloves should be worn when in contact with patients, specimens, and laboratory equipment or fixtures. When specimens are collected, gloves must be changed between every patient. In the laboratory, they are changed whenever they become noticeably contaminated or damaged and are always removed when leaving the work area. Wearing gloves is not a substitute for handwashing, and hands must be washed after gloves are removed.

A variety of gloves are available, including sterile and nonsterile, powdered and unpowdered, and latex and nonlatex. Allergy to latex is increasing among health-care workers, and laboratory personnel should be alert for symptoms of reactions associated with latex. Reactions to latex include irritant contact dermititis, which produces patches of dry, itchy irritation on the hands; delayed hypersensitivity reactions resembling poison ivy that appear 24 to 48 hours following exposure; and true, immediate hypersensitivity reactions often characterized by facial flushing and breathing difficulties. Handwashing immediately after removing gloves and avoiding powdered gloves may aid in preventing the development of latex allergies. Replacing latex gloves with nitrile or vinyl gloves provides an alternative. Any symptoms of latex allergy should be reported to a supervisor because true latex allergy can be life-threatening.⁵

Fluid-resistant laboratory coats with wrist cuffs are worn to protect clothing and skin from exposure to patients' body substances. These coats should always be completely buttoned, and gloves should be pulled over the cuffs. They are worn at all times when working with patient specimens and are removed prior to leaving the work area. They are changed when they become visibly soiled. Disposable coats are placed in containers for biohazardous waste, and nondisposable coats are placed in designated laundry receptacles.

The mucous membranes of the eyes, nose, and mouth must be protected from specimen splashes and aerosols. A variety of protective equipment is available, including goggles, full-face plastic shields, and Plexiglas countertop shields. Particular care should be taken to avoid splashes and aerosols when removing container tops, pouring specimens, and centrifuging specimens. Specimens must never be centrifuged in uncapped tubes or in uncovered centrifuges. When specimens are received in containers with contaminated exteriors, the exterior of the container must be disinfected or, if necessary, a new specimen may be requested.

Handwashing

Handwashing is emphasized in Figure 1-1 and in the Standard Precautions guidelines. Hand contact is the primary method of infection transmission. Laboratory personnel must always wash hands after gloves are removed, prior to leaving the work area, at any time when hands have been knowingly contaminated, before going to designated break areas, and before and after using bathroom facilities.

Correct handwashing technique is shown in Figure 1-2 and includes the following steps:

- 1. Wet hands with warm water.
- 2. Apply antimicrobial soap.
- 3. Rub to form a lather, create friction, and loosen debris.
- 4. Thoroughly clean between fingers, including thumbs, under fingernails and rings, and up to the wrist, for at least 15 seconds.
- 5. Rinse hands in a downward position.
- 6. Dry with a paper towel.
- 7. Turn off faucets with a clean paper towel to prevent recontamination.



Figure 1–2 Handwashing technique. (A) Wetting hands. (B) Lathering hands and creating friction. (C) Cleaning between fingers. (D) Rinsing hands. (E) Drying hands. (F) Turning off water. (From Strasinger, SK and DiLorenzo, MA: Skills for the Patient Care Technician, FA Davis, Philadelphia, 1999, p 70, with permission.)

Disposal of Biological Waste

All biological waste, except urine, must be placed in appropriate containers labeled with the biohazard symbol (see Fig. 1-1). This includes both specimens and the materials with which the specimens come in contact. The waste is then decontaminated following institutional policy: incineration, autoclaving, or pickup by a certified hazardous waste company.

Urine may be discarded by pouring it into a laboratory sink. Care must be taken to avoid splashing, and the sink should be flushed with water after specimens are discarded. Disinfection of the sink using a 1:5 or 1:10 dilution of sodium hypochlorite should be performed daily. Sodium hypochlorite dilutions stored in plastic bottles are effective for 1 month if

protected from light after preparation. The same solution also can be used for routinely disinfecting countertops and accidental spills. The solution should be allowed to air-dry on the contaminated area. Absorbent materials used for cleaning countertops and removing spills must be discarded in biohazard containers. Empty urine containers can be discarded as nonbiologically hazardous waste (Fig. 1-3).

■■● Sharp Hazards



Sharp objects in the laboratory, including needles, lancets, and broken glassware, present a serious biological hazard, particularly for the transmission of blood-borne pathogens. All sharp objects must

6 CHAPTER I • Safety in the Clinical Laboratory





Figure 1–3 Technologist disposing of urine (A) sample and (B) container.

be disposed in puncture-resistant containers. Puncture-resistant containers should be conveniently located within the work area.

■■● Chemical Hazards



The same general rules for handling biohazardous materials apply to chemically hazardous materials; that is, to avoid getting these materials in or on bodies, clothes, or work area. Every chemical in the

workplace should be presumed hazardous.

Chemical Spills

When skin contact occurs, the best first aid is to flush the area with large amounts of water for at least 15 minutes and then

seek medical attention. For this reason, all laboratory personnel should know the location and proper use of emergency showers and eye wash stations. Contaminated clothing should be removed as soon as possible. No attempt should be made to neutralize chemicals that come in contact with the skin. Chemical spill kits containing protective apparel, nonreactive absorbent material, and bags for disposal of contaminated materials should be available for cleaning up spills.

Chemical Handling

Chemicals should never be mixed together unless specific instructions are followed, and they must be added in the order specified. This is particularly important when combining acid and water. Acid should always be added to water to avoid the possibility of sudden splashing caused by the rapid generation of heat in some chemical reactions. Wearing goggles and preparing reagents under a fume hood are recommended safety precautions. Chemicals should be used from containers that are of an easily manageable size. Pipetting by mouth is unacceptable in the laboratory. State and federal regulations are in place for the disposal of chemicals and should be consulted.

Chemical Hygiene Plan

OSHA also requires all facilities that use hazardous chemicals to have a written *chemical hygiene plan* (CHP) available to employees.⁶ The purpose of the plan is to detail the following:

- 1. Appropriate work practices
- 2. Standard operating procedures
- 3. PPE
- 4. Engineering controls, such as fume hoods and flammables safety cabinets
- 5. Employee training requirements
- 6. Medical consultation guidelines

Each facility must appoint a chemical hygiene officer, who is responsible for implementing and documenting compliance with the plan. Examples of required safety equipment and information are shown in Figure 1-4.

Chemical Labeling

Hazardous chemicals should be labeled with a description of their particular hazard, such as poisonous, corrosive, or *carcinogenic*. The National Fire Protection Association (NFPA) has developed the Standard System for the Identification of the Fire Hazards of Materials, NFPA 704.⁷ This symbol system is used to inform fire fighters of the hazards they may encounter with fires in a particular area. The diamond-shaped, color-coded symbol contains information relating to health, flammability, reactivity, and personal protection/special precautions. Each category is graded on a scale of 0 to 4, based on the extent of concern. These symbols are placed on doors, cabinets, and containers. An example of this system is shown in Figure 1-5.



Figure I-4 Chemical safety aids. (A) Equipment. (B) Information and supplies. (From Strasinger, SK and DiLorenzo, MA: Skills for the Patient Care Technician, FA Davis, Philadelphia, 1999, p 70, with permission.)

HAZARDOUS MATERIALS CLASSIFICATION FIRE HAZARD **HEALTH HAZARD** Flash Point 4 Deadly 4 Below 73 F 3 Extreme Danger 3 Below 100 F 2 Hazardous 2 Below 200 F 1 Slightly Hazardous0 Normal Material 1 Above 200 F 0 Will not burn REACTIVITY **SPECIFIC HAZARD** 4 May deteriorate 3 Shock and heat OXY ACID Oxidizer may deteriorate Acid 2 Violent chemical Alkali ALK change Corrosive COR Unstable if Use No Water heated Radiation 0 0 Stable

Figure 1–5 NFPA hazardous material symbols.

Material Data Safety Sheets

The OSHA Federal Hazard Communication Standard requires that all employees have a right to know about all chemical hazards present in their workplace. The information is provided in the form of *Material Safety Data Sheets* (MSDSs) on file in the workplace. By law, vendors are required to provide these sheets to purchasers; however, the facility itself is responsible for obtaining and making MSDSs available to employees. Information contained in an MSDS includes the following:

- 1. Physical and chemical characteristics
- 2. Fire and explosion potential
- 3. Reactivity potential
- 4. Health hazards and emergency first aid procedures
- 5. Methods for safe handling and disposal

■■● Radioactive Hazards



Radioactivity is encountered in the clinical laboratory when procedures using *radioisotopes* are performed. The amount of radioactivity present in the clinical laboratory is very small and represents little

danger; however, the effects of radiation are cumulative related to the amount of exposure. The amount of radiation exposure is related to a combination of time, distance, and shielding. Persons working in a radioactive environment are required to wear measuring devices to determine the amount of radiation they are accumulating.

Laboratory personnel should be familiar with the radioactive hazard symbol shown here. This symbol must be displayed on the doors of all areas where radioactive material is present. Exposure to radiation during pregnancy presents a danger to the fetus; personnel who are pregnant or think they may be should avoid areas with this symbol.

■ ■ ● Electrical Hazards



The laboratory setting contains a large amount of electrical equipment with which workers have frequent contact. The same general rules of electrical safety observed outside the workplace apply. The

danger of water or fluid coming in contact with equipment is greater in the laboratory setting. Equipment should not be operated with wet hands. Designated hospital personnel monitor electrical equipment closely; however, laboratory personnel should continually observe for any dangerous conditions, such as frayed cords and overloaded circuits, and report them to the appropriate persons. Equipment that has become wet should be unplugged and allowed to dry completely before reusing. Equipment also should be unplugged before cleaning. All electrical equipment must be grounded with three-pronged plugs.

When an accident involving electrical shock occurs, the electrical source must be removed immediately. This must be done without touching the person or the equipment involved

Table 1-2 Types of Fires and Fire Extinguishers				
Fire Type	Extinguishing Material	Type of Fire Composition of Fire	Extinguisher	
Class A	Wood, paper, clothing	Class A	Water	
Class B	Flammable organic chemicals	Class B	Dry chemicals, carbon dioxide, foam, or halon	
Class C	Electrical	Class C	Dry chemicals, carbon dioxide, or halon	
Class D	Combustible metals	None Class ABC	Sand or dry powder Dry chemicals	
From Stracingar, SV and DiLaranza, MA: Skills for the Patient Care Technician, EA Davis, Philadelphia, 1000, p.70, with permission				

From Strasinger, SK and DiLorenzo, MA: Skills for the Patient Care Technician, FA Davis, Philadelphia, 1999, p 70, with permission.

in order to avoid transference of the current. Turning off the circuit breaker, unplugging the equipment, or moving the equipment using a nonconductive glass or wood object are safe procedures to follow.

■■● Fire/Explosive Hazards



The Joint Commission on Accreditation of Healthcare Organizations (JCAHO) requires that all health-care institutions post evacuation routes and detailed plans to follow in the event of a fire. Labo-

ratory personnel should be familiar with these procedures. When a fire is discovered, all employees are expected to take the actions in the acronym RACE:

Rescue—rescue anyone in immediate danger

Alarm—activate the institutional fire alarm system

Contain—close all doors to potentially affected areas

Extinguish—attempt to extinguish the fire, if possible; exit the area

As discussed previously, laboratory workers often use potentially volatile or explosive chemicals that require special procedures for handling and storage. Flammable chemicals should be stored in safety cabinets and explosion-proof refrigerators, and cylinders of compressed gas should be located away from heat and securely fastened to a stationary device to prevent accidental capsizing. Fire blankets may be present in the laboratory. Persons with burning clothes should be wrapped in the blanket to smother the flames.

The NFPA classifies fires with regard to the type of burning material. It also classifies the type of fire extinguisher that is used to control them. This information is summarized in Table 1–2. The multipurpose ABC fire extinguishers are the most common, but the label should always be checked before using. It is important to be able to operate the fire extinguishers. The acronym PASS can be used to remember the steps in the operation:

- 1. Pull pin
- 2. Aim at the base of the fire
- 3. Squeeze handles
- 4. Sweep nozzle side to side.

■■● Physical Hazards



Physical hazards are not unique to the laboratory, and routine precautions observed outside the work-place apply. General precautions to consider are to avoid running in rooms and hallways, watch for wet

floors, bend the knees when lifting heavy objects, keep long hair pulled back, avoid dangling jewelry, and maintain a clean, organized work area. Closed-toe shoes that provide maximum support are essential for safety and comfort.

References

- Centers for Disease Control and Prevention: Guideline for Isolation Precautions in Hospitals, Parts I and II. Web site: http://www.cdc.gov
- 2. Occupational Exposure to Blood-Borne Pathogens, Final Rule. Federal Register 29 (Dec 6), 1991.
- 3. Centers for Disease Control and Prevention. Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV and HIV and Recommendations for Post-exposure Propylaxis. MMWR June 29, 2001: 50(RR11); 1-42. Web site: http://www.cdc.gov
- 4. Centers for Disease Control and Prevention. Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposure to HIV and Recommendations for Post-exposure Prophylaxis. MMWR September 17, 2005: 54(RR09); 1-17. Web site: http://www.cdc.gov
- NIOSH Alert. Preventing Allergic Reactions to Natural Rubber Latex in the Workplace. DHHS (NIOSH) Publication 97-135. National Institute for Occupational Safety and Health, Cincinnati, Ohio, 1997.
- 6. Occupational Exposure to Hazardous Chemicals in Laboratories, Final Rule. Federal Register 55 (Jan 31), 1990.
- 7. National Fire Protection Association: Hazardous Chemical Data, No. 49. Boston, NFPA, 1991.

QUESTIONS STUDY

- **1.** In the urinalysis laboratory the primary source in the chain of infection would be:
 - A. Patients
 - B. Needlesticks
 - C. Specimens
 - D. Biohazardous waste
- 2. The best way to break the chain of infection is:
 - A. Handwashing
 - B. Personal protective equipment
 - C. Aerosol prevention
 - D. Decontamination
- **3.** Standard Precautions differ from Universal Precautions and body substance isolation by requiring:
 - A. Wearing face shields and gloves whenever blood may be encountered
 - B. Wearing gloves when encountering any moist body fluid
 - C. Washing hands after removing gloves if visual contamination is present
 - D. Wearing gloves when exposed to moist body fluids and washing hands after glove removal
- **4.** An employee who is accidentally exposed to a possible blood-borne pathogen should immediately:
 - A. Report to a supervisor
 - B. Flush the area with water
 - C. Clean the area with disinfectant
 - D. Receive HIV propylaxis
- **5**. Personnel in the urinalysis laboratory should wear lab coats that:
 - A. Do not have buttons
 - B. Are fluid-resistant
 - C. Have short sleeves
 - D. Have full-length zippers
- **6.** All of the following should be discarded in biohazardous waste containers *except*:
 - A. Urine specimen containers
 - B. Towels used for decontamination
 - C. Disposable lab coats
 - D. Blood collection tubes
- **7.** An employer who fails to provide sufficient gloves for the employees may be fined by the:
 - A. CDC
 - B. NFPA
 - C. OSHA
 - D. FDA
- **8.** An acceptable disinfectant for blood and body fluid decontamination is:
 - A. Sodium hydroxide
 - B. Antimicrobial soap
 - C. Hydrogen peroxide
 - D. Sodium hypochlorite

- **9.** Proper handwashing includes all of the following *except*:
 - A. Using warm water
 - B. Rubbing to create a lather
 - C. Rinsing hands in a downward position
 - D. Turning on the water with a paper towel
- **10**. Centrifuging an uncapped specimen may produce a biological hazard in the form of:
 - A. Vectors
 - B. Sharps contamination
 - C. Aerosols
 - D. Specimen contamination
- **11.** An employee who accidently spills acid on his arm should immediately:
 - A. Neutralize the acid with a base
 - B. Hold the arm under running water for 15 minutes
 - C. Consult the MSDSs
 - D. Wrap the arm in gauze and go to the emergency room
- **12**. When combining acid and water, ensure that:
 - A. Acid is added to water
 - B. Water is added to acid
 - C. They are added simultaneously
 - D. Water is slowly added to acid
- **13**. An employee can learn the carcinogenic potential of potassium chloride by consulting the:
 - A. Chemical hygiene plan
 - B. Material safety data sheets
 - C. OSHA standards
 - D. Urinalysis procedure manual
- **14**. Employees should not work with radioisotopes if they are:
 - A. Wearing contact lenses
 - B. Allergic to iodine
 - C. Sensitive to latex
 - D. Pregnant
- **15**. All of the following are safe to do when removing the source of an electric shock *except*:
 - A. Pulling the person away from the instrument
 - B. Turning off the circuit breaker
 - C. Using a glass container to move the instrument
 - D. Unplugging the instrument
- 16. The acronym PASS refers to:
 - A. Presence of vital chemicals
 - B. Operation of a fire extinguisher
 - C. Labeling of hazardous material
 - D. Presence of radioactive substances
- **17**. The system used by firefighters when a fire occurs in the laboratory is:
 - A. MSDS
 - B. RACE
 - C. NFPA
 - D. PASS

10 CHAPTER I • Safety in the Clinical Laboratory

Continued

- 18. A class ABC fire extinguisher contains:
 - A. Sand
 - B. Water
 - C. Dry chemicals
 - D. Acid
- 19. The first thing to do when a fire is discovered is to:
 - A. Rescue persons in danger
 - B. Activate the alarm system
 - C. Close doors to other areas
 - D. Extinguish the fire if possible
- **20**. If a red rash is observed after removing gloves, the employee:
 - A. May be washing her hands too often
 - B. May have developed a latex allergy
 - C. Should apply cortisone cream
 - D. Should not rub the hands so vigorously
- **21**. Pipetting by mouth is:
 - A. Acceptable for urine but not serum
 - B. Not acceptable without proper training
 - C. Acceptable for reagents but not specimens
 - D. Not acceptable in the laboratory

- **22**. The NPFA classification symbol contains information on all of the following *except*:
 - A. Fire hazards
 - B. Biohazards
 - C. Reactivity
 - D. Health hazards
- **23**. The classification of a fire that can be extinguished with water is:
 - A. Class A
 - B. Class B
 - C. Class C
 - D. Class D
- **24.** Employers are required to provide free immunizaton for:
 - A. HIV
 - B. HTLV-1
 - C. HBV
 - D. HCV
- 25. A possible physical hazard in the hospital is:
 - A. Wearing closed-toed shoes
 - B. Not wearing jewelry
 - C. Having short hair
 - D. Running to answer the telephone











CHAPTER 2

Renal Function

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

- Identify the components of the nephron, kidney, and excretory system.
- **2** Trace the flow of blood through the nephron and state the physiologic functions that occur.
- **3** Describe the process of glomerular ultrafiltration.
- **4** Discuss the functions and regulation of the reninangiotensin-aldosterone system.
- 5 Differentiate between active and passive transport in relation to renal concentration.
- **6** Explain the function of antidiuretic hormone in the concentration of urine.
- 7 Describe the role of tubular secretion in maintaining acid-base balance.
- 8 Identify the laboratory procedures used to evaluate glomerular filtration, tubular reabsorption and secretion, and renal blood flow.
- 9 Discuss the advantages and disadvantages in using urea, inulin, creatinine, beta₂ microglobulin, cystatin C, and radionucleotides to measure glomerular filtration.

- Given hypothetic laboratory data, calculate a creatinine clearance and determine whether the result is normal.
- 11 Discuss the clinical significance of the creatinine clearance test.
- **12** Given hypothetic laboratory data, calculate an estimated glomerular filtration rate.
- 13 Define osmolarity and discuss its relationship to urine concentration.
- **14** Describe the basic principles of clinical osmometers.
- **15** Given hypothetic laboratory data, calculate a free-water clearance and interpret the result.
- 16 Given hypothetic laboratory data, calculate a PAH clearance and relate this result to renal blood flow.
- 17 Describe the relationship of urinary ammonia and titratable acidity to the production of an acidic urine.

KEY TERMS

active transport
aldosterone
maximal reabsorptive capacity
osmolarity
passive transport

podocytes
renal threshold
renal tubular acidosis
renin
renin-angiotensin-aldosterone
system

titratable acidity tubular reabsorption tubular secretion vasopressin

12 CHAPTER 2 • Renal Function

This chapter reviews nephron anatomy and physiology and discusses their relationship to urinalysis and renal function testing. A section on laboratory assessment of renal function is included.

■■● Renal Physiology

Each kidney contains approximately 1 to 1.5 million functional units called *nephrons*. As shown in Figure 2-1, the human kidney contains two types of nephrons. Cortical nephrons, which make up approximately 85% of nephrons, are situated primarily in the cortex of the kidney. They are responsible primarily for removal of waste products and reabsorption of nutrients. Juxtamedullary nephrons have longer

loops of Henle that extend deep into the medulla of the kidney. Their primary function is concentration of the urine.

The ability of the kidneys to clear waste products selectively from the blood and simultaneously to maintain the body's essential water and electrolyte balances is controlled in the nephron by the following renal functions: renal blood flow, glomerular filtration, *tubular reabsorption*, and *tubular secretion*. The physiology, laboratory testing, and associated pathology of these four functions are discussed in this chapter.

Renal Blood Flow

The renal artery supplies blood to the kidney. The human kidneys receive approximately 25% of the blood pumped

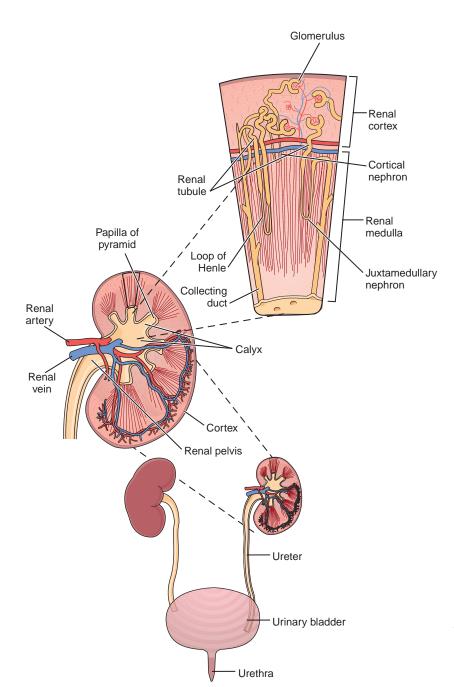


Figure 2–I The relationship of the nephron to the kidney and excretory system. (From Scanlon, VC, and Sanders, T: Essentials of Anatomy and Physiology, ed 3. FA Davis, Philadelphia, 1999, p 405, with permission.)

through the heart at all times. Blood enters the capillaries of the nephron through the *afferent arteriole*. It then flows through the glomerulus and into the *efferent arteriole*. The varying sizes of these arterioles help to create the hydrostatic pressure differential important for glomerular filtration and to maintain consistency of glomerular capillary pressure and renal blood flow within the glomerulus. Notice the smaller size of the efferent arteriole in Figure 2-2. This increases the glomerular capillary pressure.

Before returning to the renal vein, blood from the efferent arteriole enters the *peritubular capillaries* and the *vasa recta* and flows slowly through the cortex and medulla of the kidney close to the tubules. The peritubular capillaries surround the proximal and distal convoluted tubules, providing for the immediate reabsorption of essential substances from the fluid in the *proximal convoluted tubule* and final adjustment of the urinary composition in the *distal convoluted tubule*. The vasa recta are located adjacent to the ascending and descending *loop of Henle* in juxtamedullary nephrons. In this area, the major exchanges of water and salts take place between the blood and the *medullary interstitium*. This exchange maintains the *osmotic gradient* (salt concentration) in the medulla, which is necessary for renal concentration.

Based on an average body size of 1.73 m² of surface, the total renal blood flow is approximately 1200 mL/min, and the total *renal plasma flow* ranges 600 to 700 mL/min. Normal values for renal blood flow and renal function tests depend on body size. When dealing with sizes that vary greatly from the average 1.73 m² of body surface, a correction must be calculated to determine whether the observed measurements represent normal function. This calculation is covered in the discussion on tests for *glomerular filtration rate* (GFR) later

in this chapter. Variations in normal values have been published for different age groups and should be considered when evaluating renal function studies.

Glomerular Filtration

The *glomerulus* consists of a coil of approximately eight capillary lobes referred to collectively as the capillary tuft. It is located within *Bowman's capsule*, which forms the beginning of the renal tubule. Although the glomerulus serves as a nonselective filter of plasma substances with molecular weights of less than 70,000, several factors influence the actual filtration process. These include the cellular structure of the capillary walls and Bowman's capsule, *hydrostatic* and *oncotic pressures*, and the feedback mechanisms of the *reninangiotensin-aldosterone system*. Figure 2-3 provides a diagrammatic view of the glomerular areas influenced by these factors.

Cellular Structure of the Glomerulus

Plasma filtrate must pass through three cellular layers: the capillary wall membrane, the basement membrane (basal lamina), and the visceral epithelium of Bowman's capsule. The endothelial cells of the capillary wall differ from those in other capillaries by containing pores and are referred to as fenestrated. The pores increase capillary permeability but do not allow the passage of large molecules and blood cells. Further restriction of large molecules occurs as the filtrate passes through the basement membrane and the thin membranes covering the filtration slits formed by the intertwining foot processes of the *podocytes* of the inner layer of Bowman's capsule (see Fig. 2-3).

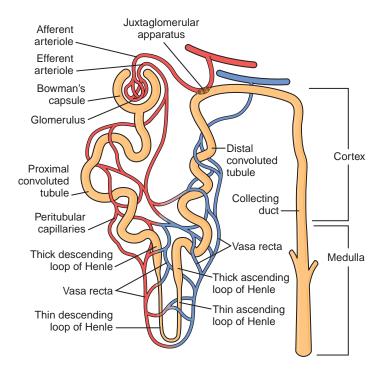
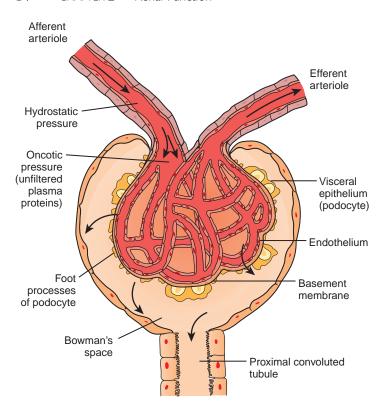


Figure 2–2 The nephron and its component parts.

14 CHAPTER 2 • Renal Function



Glomerular Pressure

As mentioned previously, the presence of hydrostatic pressure resulting from the smaller size of the efferent arteriole and the glomerular capillaries enhances filtration. This pressure is necessary to overcome the opposition of pressures from the fluid within Bowman's capsule and the oncotic pressure of unfiltered plasma proteins in the glomerular capillaries. By increasing or decreasing the size of the afferent arteriole, an autoregulatory mechanism within the juxtaglomerular apparatus maintains the glomerular blood pressure at a relatively constant rate regardless of fluctuations in systemic blood pressure. Dilation of the afferent arterioles and constriction of the efferent arterioles when blood pressure drops prevent a marked decrease in blood flowing through the kidney, thus preventing an increase in the blood level of toxic waste products. Likewise, an increase in blood pressure results in constriction of the afferent arterioles to prevent overfiltration or damage to the glomerulus.

Renin-Angiotensin-Aldosterone System

The renin-angiotensin-aldosterone system (RAAS) controls the regulation of the flow of blood to and within the glomerulus. The system responds to changes in blood pressure and plasma sodium content that are monitored by the juxtaglomerular apparatus, which consists of the juxtaglomerular cells in the afferent arteriole and the *macula densa* of the distal convoluted tubule (Fig. 2-4). Low plasma sodium content decreases water retention within the circulatory system, resulting in a decreased overall blood volume and subsequent decrease in blood pressure. When the macula densa senses such changes, a cascade of reactions within the RAAS occurs

Figure 2–3 Factors affecting glomerular filtration in the renal corpuscle.

(Fig. 2-5). **Renin**, an enzyme produced by the juxtaglomerular cells, is secreted and reacts with the blood-borne substrate angiotensinogen to produce the inert hormone angiotensin I. As angiotensin I passes through the lungs, angiotensin converting enzyme (**ACE**) changes it to the active form angiotensin II. Angiotensin II corrects renal blood flow in the following ways: causing vasodilation of the afferent arterioles and constriction of the efferent arterioles, stimulating reabsorption of sodium in the proximal convoluted tubules, and triggering the release of the sodium-retaining hormone **aldosterone** by the adrenal cortex and antidiuretic hormone by the hypothalmus (Table 2–1). As systemic blood pressure and plasma sodium content increase, the secretion of renin decreases. Therefore, the actions of angiotensin II produce a constant pressure within the nephron.

As a result of the above glomerular mechanisms, every minute approximately two to three million glomeruli filter approximately 120 mL of water-containing low-molecular-weight substances. Because this filtration is nonselective, the only difference between the compositions of the filtrate and the plasma is the absence of plasma protein, any protein-bound substances, and cells. Analysis of the fluid as it leaves the glomerulus shows the filtrate to have a specific gravity of 1.010 and confirms that it is chemically an ultrafiltrate of plasma. This information provides a useful baseline for evaluating the renal mechanisms involved in converting the plasma ultrafiltrate into the final urinary product.

Tubular Reabsorption

The body cannot lose 120 mL of water-containing essential substances every minute. Therefore, when the plasma ultrafil-

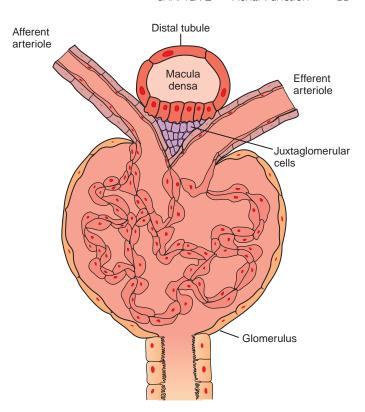


Figure 2–4 Close contact of the distal tubule with the afferent arteriole, macula densa, and the juxtaglomerular cells within the juxtaglomerular apparatus.

trate enters the proximal convoluted tubule, the nephrons, through cellular transport mechanisms, begin reabsorbing these essential substances and water (Table 2–2).

Reabsorption Mechanisms

The cellular mechanisms involved in tubular reabsorption are termed *active* and *passive transport*. For active transport to occur, the substance to be reabsorbed must combine with a carrier protein contained in the membranes of the renal

tubular cells. The electrochemical energy created by this interaction transfers the substance across the cell membranes and back into the bloodstream. Active transport is responsible for the reabsorption of glucose, amino acids, and salts in the proximal convoluted tubule, chloride in the ascending loop of Henle, and sodium in the distal convoluted tubule.

Passive transport is the movement of molecules across a membrane as a result of differences in their concentration or electrical potential on opposite sides of the membrane. These

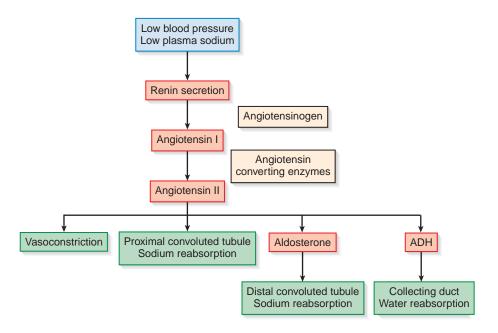


Figure 2–5 Algorithm of the reninangiotensin-aldosterone system.

Table 2-1 **Actions of the RAAS**

- 1. Dilation of the afferent arteriole and constriction of the efferent arteriole
- 2. Stimulation of sodium reabsorption in the proximal convoluted tubule
- 3. Triggers the adrenal cortex to release the sodiumretaining hormone, aldosterone, to cause reabsorption of sodium and excretion of potassium in the distal convoluted tubule and collecting duct
- 4. Triggers release of antidiuretic hormone by the hypothalmus to stimulate water reabsorption in the collecting duct

physical differences are called gradients. Passive reabsorption of water takes place in all parts of the nephron except the ascending loop of Henle, the walls of which are impermeable to water. Urea is passively reabsorbed in the proximal convoluted tubule and the ascending loop of Henle, and passive reabsorption of sodium accompanies the active transport of chloride in the ascending loop.

Active transport, like passive transport, can be influenced by the concentration of the substance being transported. When the plasma concentration of a substance that is normally completely reabsorbed reaches an abnormally high level, the filtrate concentration exceeds the maximal reabsorptive capacity (Tm) of the tubules, and the substance begins appearing in the urine. The plasma concentration at which active transport stops is termed the renal threshold. For glucose, the renal threshold is 160 to 180 mg/dL, and glucose appears in the urine when the plasma concentration reaches this level. Knowledge of the renal threshold and the plasma concentration can be used to distinguish between excess solute filtration and renal tubular damage. For exam-

Table 2-2 Tubular Reabsorption			
	Substance	Location	
Active transport	Glucose, amino acids, salts	Proximal con- voluted tubule	
	Chloride	Ascending loop of Henle	
	Sodium	Proximal and distal convoluted tubules	
Passive transport	Water	Proximal convoluted tubule, descending loop of Henle, and collecting duct	
	Urea	Proximal convoluted tubule and ascending loop of Henle	
	Sodium	Ascending loop of Henle	

ple, glucose appearing in the urine of a person with a normal blood glucose level is the result of tubular damage and not diabetes mellitus.

Active transport of more than two-thirds of the filtered sodium out of the proximal convoluted tubule is accompanied by the passive reabsorption of an equal amount of water. Therefore, as can be seen in Figure 2-6, the fluid leaving the proximal convoluted tubule still maintains the same concentration as the ultrafiltrate.

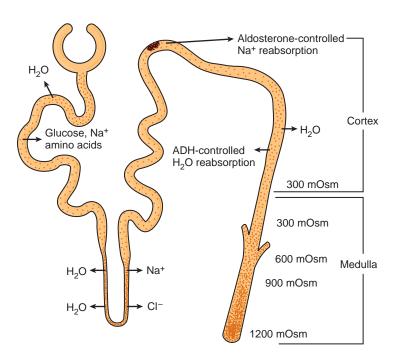


Figure 2–6 Renal concentration.

Tubular Concentration

Renal concentration begins in the descending and ascending loops of Henle, where the filtrate is exposed to the high osmotic gradient of the renal medulla. Water is removed by osmosis in the descending loop of Henle, and sodium and chloride are reabsorbed in the ascending loop. Excessive reabsorption of water as the filtrate passes through the highly concentrated medulla is prevented by the water-impermeable walls of the ascending loop. This selective reabsorption process is called the *countercurrent mechanism* and serves to maintain the osmotic gradient of the medulla. The sodium and chloride leaving the filtrate in the ascending loop prevent dilution of the medullary interstitium by the water reabsorbed from the descending loop. Maintenance of this osmotic gradient is essential for the final concentration of the filtrate when it reaches the *collecting duct*.

In Figure 2-6, the actual concentration of the filtrate leaving the ascending loop is quite low owing to the reabsorption of salt and not water in that part of the tubule. Reabsorption of sodium continues in the distal convoluted tubule, but it is now under the control of the hormone aldosterone, which regulates reabsorption in response to the body's need for sodium (see Fig. 2-5).

Collecting Duct Concentration

The final concentration of the filtrate through the reabsorption of water begins in the late distal convoluted tubule and continues in the collecting duct. Reabsorption depends on the osmotic gradient in the medulla and the hormone <code>vasopressin</code> (antidiuretic hormone <code>[ADH]</code>). One would expect that as the dilute filtrate in the collecting duct comes in contact with the higher osmotic concentration of the

medullary interstitium, passive reabsorption of water would occur. However, the process is controlled by the presence or absence of ADH, which renders the walls of the distal convoluted tubule and collecting duct permeable or impermeable to water. A high level of ADH increases permeability, resulting in increased reabsorption of water, and a low-volume concentrated urine. Likewise, absence of ADH renders the walls impermeable to water, resulting in a large volume of dilute urine. Just as the production of aldosterone is controlled by the body's sodium concentration, production of ADH is determined by the state of body hydration. Therefore, the chemical balance in the body is actually the final determinant of urine volume and concentration. The concept of ADH control can be summarized in the following manner:

 \uparrow Body Hydration = \downarrow ADH = \uparrow Urine Volume \downarrow Body Hydration = \uparrow ADH = \downarrow Urine Volume

Tubular Secretion

In contrast to tubular reabsorption, in which substances are removed from the glomerular filtrate and returned to the blood, tubular secretion involves the passage of substances from the blood in the peritubular capillaries to the tubular filtrate (Fig. 2-7). Tubular secretion serves two major functions: elimination of waste products not filtered by the glomerulus and regulation of the acid-base balance in the body through the secretion of hydrogen ions.

Many foreign substances, such as medications, cannot be filtered by the glomerulus because they are bound to plasma proteins. However, when these protein-bound substances enter the peritubular capillaries, they develop a stronger affin-

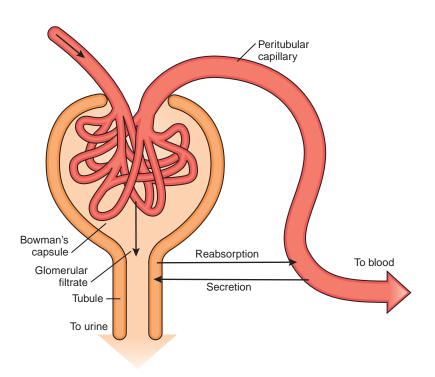


Figure 2–7 The movement of substances in the nephron.

18 CHAPTER 2 • Renal Function

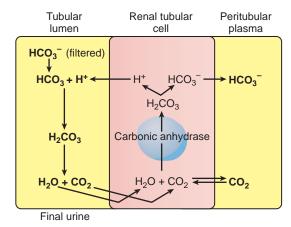


Figure 2–8 Reabsorption of filtered bicarbonate.

ity for the tubular cells and dissociate from their carrier proteins, which results in their transport into the filtrate by the tubular cells. The major site for removal of these nonfiltered substances is the proximal convoluted tubule.

Acid-Base Balance

To maintain the normal blood pH of 7.4, the blood must buffer and eliminate the excess acid formed by dietary intake and body metabolism. The buffering capacity of the blood depends on bicarbonate (HCO₃⁻) ions, which are readily filtered by the glomerulus and must be expediently returned to the blood to maintain the proper pH. As shown in Figure 2-8, the secretion of hydrogen ions (H⁺) by the renal tubular cells into the filtrate prevents the filtered bicarbonate from being excreted in the urine and causes the return of a bicarbonate ion to the plasma. This process provides for almost 100% reabsorption of filtered bicarbonate and occurs primarily in the proximal convoluted tubule.

As a result of their small molecular size, hydrogen ions are readily filtered and reabsorbed. Therefore, the actual excretion of excess hydrogen ions also depends on tubular secretion. Figures 2-9 and 2-10 are diagrams of the two primary methods for hydrogen ion excretion in the urine. In Figure 2-9 the secreted hydrogen ion combines with a filtered phosphate ion instead of a bicarbonate ion and is excreted rather than reabsorbed. Additional excretion of hydrogen ions is accomplished through their reaction with ammonia produced and secreted by the cells of the distal convoluted tubule. In the proximal convoluted tubule, ammonia is produced from the breakdown of the amino acid glutamine. The ammonia reacts with the $\rm H^+$ to form the ammonium ion $\rm (NH_4^{\ +})$ (see Fig. 2-10). The resulting ammonium ion is excreted in the urine.

All three of these processes occur simultaneously at rates determined by the acid-base balance in the body. A disruption in these secretory functions can result in *metabolic acidosis* or *renal tubular acidosis*, the inability to produce an acid urine.

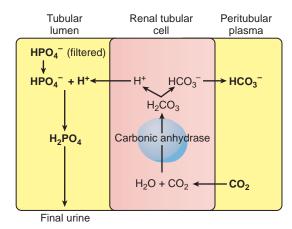


Figure 2–9 Excretion of secreted hydrogen ions combined with phosphate.

Renal Function Tests

This brief review of renal physiology shows that there are many metabolic functions and chemical interactions to be evaluated through laboratory tests of renal function. In Figure 2-11, the parts of the nephron are related to the laboratory tests used to assess their function.

Glomerular Filtration Tests

The standard test used to measure the filtering capacity of the glomeruli is the clearance test. As its name implies, a clearance test measures the rate at which the kidneys are able to remove (to clear) a filterable substance from the blood. To ensure that glomerular filtration is being measured accurately, the substance analyzed must be one that is neither reabsorbed nor secreted by the tubules. Other factors to consider in the selection of a clearance test substance include the stability of the substance in urine during a possible 24-hour collection period, the consistency of the plasma level, the substance's availability to the body, and the availability of tests for analysis of the substance.

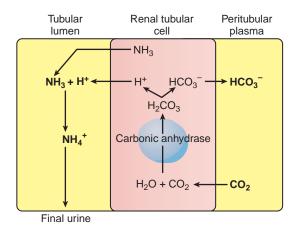


Figure 2–10 Excretion of secreted hydrogen ions combined with ammonia produced by the tubules.

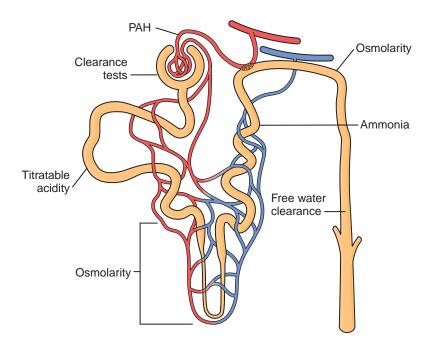


Figure 2–II The relationship of nephron areas to renal function tests.

Clearance Tests

The earliest glomerular filtration tests measured urea because of its presence in all urine specimens and the existence of routinely used methods of chemical analysis. Because approximately 40% of the filtered urea is reabsorbed, normal values were adjusted to reflect the reabsorption, and patients **were** hydrated to produce a urine flow of 2 mL/min to ensure that no more than 40% of the urea was reabsorbed. At present, the use of urea as a test substance for glomerular filtration has been replaced by the measurement of other substances including *creatinine*, *inulin*, *beta*₂ *microglobulin*, cystatin C, or radioisotopes. Each procedure has its advantages and disadvantages.

Inulin Clearance

Inulin, a polymer of fructose, is an extremely stable substance that is not reabsorbed or secreted by the tubules. It is not a normal body constituent, however, and must be infused at a constant rate throughout the testing period. A test that requires an infused substance is termed an *exogenous procedure* and is seldom the method of choice if a suitable test substance is already present in the body *(endogenous procedure)*. Therefore, although inulin was the original reference method for clearance tests, it is currently not used for glomerular filtration testing.

Creatinine Clearance

Currently, routine laboratory measurements of GFR employ creatinine as the test substance. Creatinine, a waste product of muscle metabolism that is normally found at a relatively constant level in the blood, provides the laboratory with an endogenous procedure for evaluating glomerular function. The use of creatinine has several disadvantages not found with inulin, and careful consideration should be given to them. They are as follows:

- 1. Some creatinine is secreted by the tubules, and secretion increases as blood levels rise.
- 2. Chromogens present in human plasma react in the chemical analysis. Their presence, however, may help counteract the falsely elevated rates caused by tubular secretion.
- 3. Medications, including gentamicin, cephalosporins, and cimetidine (Tagamet), inhibit tubular secretion of creatinine, thus causing falsely low serum levels.¹
- 4. Bacteria will break down urinary creatinine if specimens are kept at room temperature for extended periods.²
- A diet heavy in meat consumed during collection of a 24-hour urine specimen will influence the results if the plasma specimen is drawn prior to the collection period.
- Measurement of *creatinine clearance* is not a reliable indicator in patients suffering from muscle-wasting diseases.

Because of these drawbacks, abnormal results may be followed by more sophisticated tests, but the creatinine clearance test provides the clinical laboratory with a method for screening the GFR.

Procedure

By far the greatest source of error in any clearance procedure utilizing urine is the use of improperly timed urine specimens. The importance of using an accurately timed specimen (see Chapter 3) will become evident in the following discussion of the calculations involved in converting isolated laboratory measurements to GFR. The GFR is reported in mL/min; therefore, determining the number of milliliters of plasma from which the clearance substance (creatinine) is completely removed during 1 minute is necessary. To calculate this infor-

20 CHAPTER 2 • Renal Function

mation, one must know urine volume in mL/min (V), urine creatinine concentration in mg/dL (U), and plasma creatinine concentration in mg/dL (P).

The urine volume is calculated by dividing the number of milliliters in the specimen by the number of minutes used to collect the specimen.

Example

Calculate the urine volume (V) for a 2-hour specimen measuring 240 mL:

2 hours \times 60 minutes = 120 minutes 240 mL/120 minutes = 2 mL/min

V = 2 mL/min

The plasma and urine concentrations are determined by chemical testing. The standard formula used to calculate the milliliters of plasma cleared per minute (*C*) is:

$$C = \frac{UV}{P}$$

This formula is derived as follows. The milliliters of plasma cleared per minute (C) times the mg/dL of plasma creatinine (P) must equal the mg/dL of urine creatinine (U) times the urine volume in mL/min (V), because all of the filtered creatinine will appear in the urine. Therefore:

$$CP = UV \text{ and } C = \frac{UV}{P}$$

Example

Using urine creatinine of 120 mg/dL (U), plasma creatinine of 1.0 mg/dL (P), and urine volume of 1440 mL obtained from a 24-hour specimen (V), calculate the GFR.

$$V = \frac{1440 \text{ mL}}{60 \text{ minutes} \times 24 = 1440 \text{ minutes}} = 1 \text{ mL/min}$$

$$C = \frac{120 \text{ mg/dL (U)} \times 1 \text{ mL/min (V)}}{1.0 \text{ mg/dL (P)}} = 120 \text{ mL/min}$$

By analyzing this calculation and referring to Figure 2-12, at a 1 mg/dL concentration, each milliliter of plasma contains 0.01 mg creatinine. Therefore, to arrive at a urine concentration of 120 mg/dL (1.2 mg/mL), it is necessary to clear 120 mL of plasma. Although the filtrate volume is reduced, the amount of creatinine in the filtrate does not change because the creatinine is not reabsorbed. Knowing that in the average person (1.73 m² body surface) the approximate amount of plasma filtrate produced per minute is 120 mL, it is not surprising that normal creatinine clearance values approach 120 mL/min (men, 107 to 139 mL/min; women, 87 to 107 mL/min). The normal plasma creatinine is 0.5 to 1.5 mg/dL. These normal values take into

account variations in size and muscle mass. Values are considerably lower in older people, however, and an adjustment may also have to be made to the calculation when dealing with body sizes that deviate greatly from 1.73 m² of surface, such as with children. To adjust a clearance for body size, the formula is:

$$C = \frac{UV}{P} \times \frac{1.73}{A}$$

with A being the actual body size in square meters of surface. The actual body size may be calculated as:

$$log A = (0.425 \times log weight) +$$

$$(0.725 \times log height) -2.144$$

or it may be obtained from the nomogram shown in Figure 2-13.

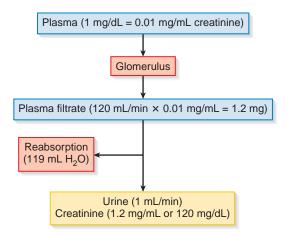


Figure 2–I2 A diagram representing creatinine filtration and excretion.

Clinical Significance

When interpreting the results of a creatinine clearance test, the GFR is determined not only by the number of functioning nephrons but also by the functional capacity of these nephrons. In other words, even though half of the available nephrons may be nonfunctional, a change in the GFR will not occur if the remaining nephrons double their filtering capacity. This is evidenced by persons who lead normal lives with only one kidney. Therefore, although the creatinine clearance is a frequently requested laboratory procedure, its value does not lie in the detection of early renal disease. Instead, it is used to determine the extent of nephron damage in known cases of renal disease, to monitor the effectiveness of treatment designed to prevent further nephron damage, and to determine the feasibility of administering medications, which can build up to dangerous blood levels if the GFR is markedly reduced.

Calculated Glomerular Filtration Estimates

Formulas have been developed to provide estimates of the GFR based on the serum creatinine without the urine creati-

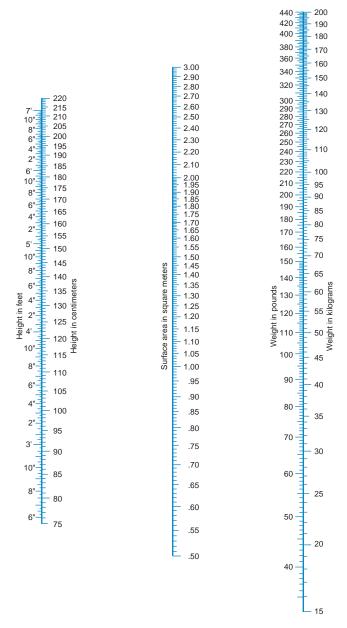


Figure 2–13 A nomogram for the determination of body surface area. (From Boothby, WM, and Sandiford, RB: Nomogram for determination of body surface area. N Engl J Med 185:227, 1921, with permission.)

nine. These formulas are becoming more frequently used in clinical medicine. As discussed, the creatinine clearance is not useful in detecting early renal disease. Therefore the calculated clearances are being used for monitoring patients already diagnosed with renal disease or at risk for renal disease. In addition, the formulas are valuable when medications that require adequate renal clearance need to be prescribed.

The most frequently used formula was developed by Cockcroft and Gault.³ It is also used to document eligibility for reimbursement by the Medicare End Stage Renal Disease Program and for evaluating patient placement on kidney transplant lists.⁴ Variables included in the original formula are age, sex, and body weight in kilograms.

$$C_{\rm cr} = \frac{(140 - age)(\text{weight in kilograms})}{72 \times \text{serum creatinine in mg/dL}}$$

The results are multiplied by 0.85 for female patients. Modifications to the original formula substitute *ideal body weight* in kilograms and adjusted body weight in kilograms. This is done to correct for weight that may not be the result of muscle mass, i.e., fatty tissue. The calculation for ideal body weight (IBW) is:

Males: 50 kg + 2.3 kg for each inch of height over 60 inches

Females: 45.5 kg + 2.3 kg for each inch of height over 60 inches

The calculation for adjusted body weight (AjBW) is:

$$IBW + 0.3 (ABW-IBW)$$

A newer formula, called the Modification of Diet in Renal Disease (MDRD) system, utilizes additional variables and does not include body weight. The variables include ethnicity, blood urea nitrogen, and serum albumin. Several variations of the formula are available, utilizing one or more of the additional variables. An example of the MDRD study formula is:

GFR= 170
$$\times$$
 serum creatinine^{-0.999} \times age^{-0.176} \times 0.822 (if patient is female) \times 1.1880 (if patient is black) \times BUN^{-0.170} \times serum albumin^{+0.318}

A laboratory advantage of this formula is that, as body weight is omitted, all results are available from the laboratory computer information, and the calculation can be performed automatically by the instrument performing the serum creatinine.⁴

The development of simplified procedures measuring the plasma disappearance of infused substances, thereby eliminating the need for urine collection, has enhanced interest in exogenous procedures. Injection of radionucleotides such as ¹²⁵I-iothalamate provides a method for determining glomerular filtration through the plasma disappearance of the radioactive material and enables visualization of the filtration in one or both kidneys.⁵

Good correlation between the GFR and plasma levels of beta₂ microglobulin has been demonstrated. Beta₂ microglobulin (molecular weight 11,800) dissociates from human leukocyte antigens at a constant rate and is rapidly removed from the plasma by glomerular filtration. Sensitive methods using enzyme immunoassay are available for the measurement of beta₂ microglobulin.⁶ A rise in the plasma level of beta₂ microglobulin has been shown to be a more sensitive indicator of a decrease in GFR than creatinine clearance. However, the test is not reliable in patients who have a history of immunologic disorders or malignancy.⁷

Another serum marker that can be used to monitor GFR is cystatin *C*. *Cystatin C* is a small protein (molecular weight 13,359) produced at a constant rate by all nucleated cells. It is readily filtered by the glomerulus and reabsorbed and broken down by the renal tubular cells. Therefore, no cystatin *C* is secreted by the tubules, and the serum concentration can

22 CHAPTER 2 • Renal Function

be directly related to the GFR. Immunoassay procedures are available for measuring cystatin C.8 Monitoring levels of cystatin C is recommended for pediatric patients, persons with diabetes, the elderly, and critically ill patients.9

Tubular Reabsorption Tests

Whereas measurement of the GFR is not a useful indication of early renal disease, the loss of tubular reabsorption capability is often the first function affected in renal disease. This is not surprising when one considers the complexity of the tubular reabsorption process.

Tests to determine the ability of the tubules to reabsorb the essential salts and water that have been nonselectively filtered by the glomerulus are called concentration tests. As mentioned, the ultrafiltrate that enters the tubules has a specific gravity of 1.010; therefore, after reabsorption one would expect the final urine product to be more concentrated. However, as you perform routine urinalysis, you will see that many specimens do not have a specific gravity higher than 1.010, yet no renal disease is present. This is because urine concentration is largely determined by the body's state of hydration, and the normal kidney will reabsorb only the amount of water necessary to preserve an adequate supply of body water.

As can be seen in Figure 2-14, both specimens contain the same amount of solute; however, the urine density (specific gravity) of patient A will be higher. Therefore, control of fluid intake must be incorporated into laboratory tests that measure the concentrating ability of the kidney.

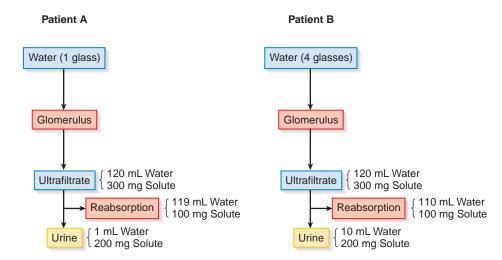
Throughout the years, various methods have been used to produce water deprivation, including the Fishberg and Mosenthal concentration tests, which measured specific gravity. In the Fishberg test, patients were deprived of fluids for 24 hours prior to measuring specific gravity. The Mosenthal test compared the volume and specific gravity of day and night urine samples to evaluate concentrating ability. Neither test is used now because the information provided by specific

gravity measurements is most useful as a screening procedure, and quantitative measurement of renal concentrating ability is best assessed through osmometry. However, persons with normal concentrating ability should have a specific gravity of 1.025 when deprived of fluids for 16 hours. Following overnight water deprivation, a urine *osmolarity* of 800 mOsm or above indicates normal concentrating ability.

Osmolarity

Specific gravity depends on the number of particles present in a solution and the density of these particles, whereas osmolarity is affected only by the number of particles present. When evaluating renal concentration ability, the substances of interest are small molecules, primarily sodium (molecular weight, 23) and chloride (molecular weight, 35.5). However, urea (molecular weight, 60), which is of no importance to this evaluation, will contribute more to the specific gravity than will the sodium and chloride molecules. Because all three molecules contribute equally to the osmolarity of the specimen, a more representative measure of renal concentrating ability can be obtained by measuring osmolarity.

An osmole is defined as 1 g molecular weight of a substance divided by the number of particles into which it dissociates. A nonionizing substance such as glucose (molecular weight, 180) contains 180 g per osmole, whereas sodium chloride (NaCl) (molecular weight, 58.5), if completely dissociated, contains 29.25 g per osmole. Just like molality and molarity, there are osmolality and osmolarity. An osmolal solution of glucose has 180 g of glucose dissolved in 1 kg of solvent. An osmolar solution of glucose has 180 g of glucose dissolved in 1 L of solvent. In the clinical laboratory, the terms are used interchangeably, inasmuch as the difference in normal temperature conditions with water as the solvent is minimal. The unit of measure used in the clinical laboratory is the milliosmole (mOsm), because it is not practical when dealing with body fluids to use a measurement as large as the osmole (23 g of sodium per liter or kilogram).



Specific gravity 1.015

Specific gravity 1.005

Figure 2–14 The effect of hydration on renal concentration.

The osmolarity of a solution can be determined by measuring a property that is mathematically related to the number of particles in the solution (colligative property) and comparing this value with the value obtained from the pure solvent. Solute dissolved in solvent causes the following changes in colligative properties: lower freezing point, higher boiling point, increased osmotic pressure, and lower vapor pressure.

Because water is the solvent in both urine and plasma, the number of particles present in a sample can be determined by comparing a colligative property value of the sample with that of pure water. Clinical laboratory instruments are available to measure freezing point depression and vapor pressure depression.

Freezing Point Osmometers

Measurement of freezing point depression was the first principle incorporated into clinical osmometers, and many instruments employing this technique are available. These osmometers determine the freezing point of a solution by supercooling a measured amount of sample to approximately 27°C. The supercooled sample is vibrated to produce crystallization of water in the solution. The heat of fusion produced by the crystallizing water temporarily raises the temperature of the solution to its freezing point. A temperature-sensitive probe measures this temperature increase, which corresponds to the freezing point of the solution, and the information is converted into milliosmoles. Conversion is made possible by the fact that 1 mol (1000 mOsm) of a nonionizing substance dissolved in 1 kg of water is known to lower the freezing point 1.86°C. Therefore, by comparing the freezing point depression of an unknown solution with that of a known molal solution, the osmolarity of the unknown solution can be calculated. Clinical osmometers use solutions of known NaCl concentration as their reference standards because a solution of partially ionized substances is more representative of urine and plasma composition.

Vapor Pressure Osmometers

The other instrument used in clinical osmometry is called the vapor pressure osmometer. The actual measurement performed, however, is that of the dew point (temperature at which water vapor condenses to a liquid). The depression of dew point temperature by solute parallels the decrease in vapor pressure, thereby providing a measure of this colligative property.

Samples are absorbed into small filter paper disks that are placed in a sealed chamber containing a temperature-sensitive thermocoupler. The sample evaporates in the chamber, forming a vapor. When the temperature in the chamber is lowered, water condenses in the chamber and on the thermocoupler. The heat of condensation produced raises the temperature of the thermocoupler to the dew point temperature. This dew point temperature is proportional to the vapor pressure from the evaporating sample. Temperatures are compared with those of the NaCl standards and converted into

milliosmoles. The vapor pressure osmometer uses microsamples of less than 0.01 mL; therefore, care must be taken to prevent any evaporation of the sample prior to testing. Correlation studies have shown more variation with vapor pressure osmometers, stressing the necessity of careful technique.

Technical Factors

Factors to consider because of their influence on true osmolarity readings include lipemic serum, lactic acid, and volatile substances, such as ethanol, in the specimen. In lipemic serum, the displacement of serum water by insoluble lipids produces erroneous results with both vapor pressure and freezing point osmometers. Falsely elevated values owing to the formation of lactic acid also occur with both methods if serum samples are not separated or refrigerated within 20 minutes. Vapor pressure osmometers do not detect the presence of volatile substances, such as alcohol, as they become part of the solvent phase; however, measurements performed on similar specimens using freezing point osmometers will be elevated.

Clinical Significance

Major clinical uses of osmolarity include initially evaluating renal concentrating ability, monitoring the course of renal disease, monitoring fluid and electrolyte therapy, establishing the differential diagnosis of *hypernatremia* and *hyponatremia*, and evaluating the secretion of and renal response to ADH. These evaluations may require determination of serum in addition to urine osmolarity.

Normal serum osmolarity values are from 275 to 300 mOsm. Normal values for urine osmolarity are difficult to establish, because factors such as fluid intake and exercise can greatly influence the urine concentration. Values can range from 50 to 1400 mOsm.² Determining the ratio of urine to serum osmolarity can provide a more accurate evaluation. Under normal random conditions, the ratio of urine to serum osmolarity should be at least 1:1; after controlled fluid intake, it should reach 3:1.

The ratio of urine to serum osmolarity, in conjunction with procedures such as controlled fluid intake and injection of ADH, is used to differentiate whether diabetes insipidus is caused by decreased ADH production or inability of the renal tubules to respond to ADH. Failure to achieve a ratio of 3:1 following injection of ADH indicates that the collecting duct does not have functional ADH receptors. In contrast, if concentration takes place following ADH injection, an inability to produce adequate ADH is indicated. Tests to measure the ADH concentration in plasma and urine directly are available for difficult diagnostic cases. ¹⁰

Free Water Clearance

The ratio of urine to serum osmolarity can be further expanded by performing the analyses using water deprivation and a timed urine specimen and calculating the *free water clearance*. The free water clearance is determined by first

24 CHAPTER 2 • Renal Function

calculating the *osmolar clearance* using the standard clearance formula:

$$C_{\text{osm}} = \frac{U_{\text{osm}} \times V}{P_{\text{osm}}}$$

and then subtracting the osmolar clearance value from the urine volume in mL/min.

Example

Using a urine osmolarity of 600 mOsm (U), a urine volume of 2 mL/min (V), and a plasma osmolarity of 300 mOsm (P), calculate the free water clearance:

$$C_{\text{osm}} = \frac{600 \text{ (U)} \times 2 \text{ (V)}}{300 \text{ (P)}} = 4.0 \text{ mL/min}$$

$$C_{H_2O} = 2$$
 (V) - 4.0 (C_{osm}) = -2.0 (free water clearance)

Calculation of the osmolar clearance indicates how much water must be cleared each minute to produce a urine with the same osmolarity as the plasma. The ultrafiltrate contains the same osmolarity as the plasma; therefore, the osmotic differences in the urine are the result of renal concentrating and diluting mechanisms. By comparing the osmolar clearance with the actual urine volume excreted per minute, it can be determined whether the water being excreted is more or less than the amount needed to maintain an osmolarity the same as that of the ultrafiltrate.

The above calculation shows a free water clearance of -2.0, indicating that less than the necessary amount of water is being excreted, a possible state of dehydration. If the value had been 0, no renal concentration or dilution would be taking place; likewise, if the value had been +2.0, excess water would have been excreted. Therefore, calculation of the free water clearance is used to determine the ability of the kidney to respond to the state of body hydration.

Tubular Secretion and Renal Blood Flow Tests

Tests to measure tubular secretion of nonfiltered substances and renal blood flow are closely related in that total renal blood flow through the nephron must be measured by a substance that is secreted rather than filtered through the glomerulus. Impaired tubular secretory ability or inadequate presentation of the substance to the capillaries owing to decreased renal blood flow may cause an abnormal result. Therefore, an understanding of the principles and limitations of the tests and correlation with other clinical data is important in test interpretation.

The test most commonly associated with tubular secretion and renal blood flow is the p-aminohippuric acid (PAH) test. Historically, excretion of the dye phenolsulfon-phthalein (PSP) was used to evaluate these functions. Standardization and interpretation of PSP results are difficult,

however, because of interference by medications and elevated waste products in patients' serum and the necessity to obtain several very accurately timed urine specimens. Therefore, the PSP test is not currently performed.

PAH Test

To measure the exact amount of blood flowing through the kidney, it is necessary to use a substance that is completely removed from the blood (plasma) each time it comes in contact with functional renal tissue. The principle is the same as in the clearance test for glomerular filtration. However, to ensure measurement of the blood flow through the entire nephron, the substance must be removed from the blood primarily in the peritubular capillaries rather than being removed when the blood reaches the glomerulus.

Although it has the disadvantage of being exogenous, the chemical PAH meets the criteria needed to measure renal blood flow. This nontoxic substance is loosely bound to plasma proteins, which permits its complete removal as the blood passes through the peritubular capillaries. Except for a small amount of PAH contained in plasma that does not come in contact with functional renal tissue, all the plasma PAH is secreted by the proximal convoluted tubule. Therefore, the volume of plasma flowing through the kidneys determines the amount of PAH excreted in the urine. The standard clearance formula

$$C_{PAH} (mL/min) = \frac{U (mg/dL PAH) \times V (mL/min urine)}{P (mg/dL PAH)}$$

can be used to calculate the effective renal plasma flow. Based on normal hematocrit readings, normal values for the effective renal plasma flow range from 600 to 700 mL/min, making the average renal blood flow about 1200 mL/min. The actual measurement is renal plasma flow rather than renal blood flow, because the PAH is contained only in the plasma portion of the blood. Also, the term "effective" is included because approximately 8% of the renal blood flow does not come into contact with the functional renal tissue. 11

The amount of PAH infused must be monitored carefully to ensure accurate results; therefore, the test is usually performed by specialized renal laboratories. Nuclear medicine procedures using radioactive hippurate can determine renal blood flow by measuring the plasma disappearance of a single radioactive injection and at the same time provide visualization of the blood flowing through the kidneys.⁵

Titratable Acidity and Urinary Ammonia

The ability of the kidney to produce an acid urine depends on the tubular secretion of hydrogen ions and production and secretion of ammonia by the cells of the distal convoluted tubule. A normal person excretes approximately 70 mEq/day of acid in the form of either titratable acid (H+), hydrogen phosphate ions (H₂PO₄⁻), or ammonium ions (NH₄+). In normal persons, a diurnal variation in urine acidity consisting of alkaline tides appears shortly after arising and postprandially at approximately 2 p.m. and 8 p.m. The lowest pH is found at night.

The inability to produce an acid urine in the presence of metabolic acidosis is called renal tubular acidosis. This condition may result from impaired tubular secretion of hydrogen ions associated with the proximal convoluted tubule or defects in ammonia secretion associated with the distal convoluted tubule.

Measurement of urine pH, *titratable acidity*, and urinary ammonia can be used to determine the defective function. The tests can be run simultaneously on either fresh or toluene-preserved urine specimens collected at 2-hour intervals from patients who have been primed with an acid load consisting of oral ammonium chloride. By titrating the amount of free H+ (titratable acidity) and then the total acidity of the specimen, the ammonium concentration can be calculated as the difference between the titratable acidity and the total acidity.

References

- 1. Berger, A: Renal function and how to assess it. Brit J Med 321:1444, 2000.
- 2. Pincus, MR, Preuss, HG, and Henry, JB: Evaluation of renal function and water, electrolyte and acid-base balance. In Henry, JB (ed): Clinical Diagnosis and Management by Laboratory Methods. WB Saunders, Philadelphia, 1996.
- Cockcroft, DW, and Gault, HH: Prediction of creatinine clearance from serum creatinine. N Engl J Med 281:1405-1415, 1969.
- 4. Levey, AS, et al: A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. Ann Intern Med 130(6):461-470, 1999.
- Chachati, A, et al: Rapid method for the measurement of differential renal function: Validation. J Nucl Med 28(5): 829-836, 1987.
- Peterson, L: Beta₂ microglobulin. Clin Chem News 14(1):6, 1988.
- 7. Murray, B, and Ferris, TF: Blood and urinary chemistries in the evaluation of renal function. Sem Nephrol 5(3):208-221, 1985.
- 8. Laterza, OE, Price, CP, and Scott, MG: Cystatin C: An improved estimator of glomerular filtration rate? Clin Chem 48(5):699-707, 2002.
- 9. Tan, GS, et al: Clinical usefulness of cystatin C for the estimation of glomerular filtration rate in type 1 diabetes. Crit Care 9(2):139-143, 2005.
- 10. Daves, BB, and Zenser, TV: Evaluation of renal concentrating and diluting ability. Clin Lab Med 13(1):131-134, 1993.
- 11. Duston, H, and Corcoran, A: Functional interpretation of renal tests. Med Clin North Am 39:947-956, 1955.

QUESTIONS STUDY

- **1**. The type of nephron responsible for renal concentration is the:
 - A. Cortical
 - B. Juxtaglomerular
- 2. The function of the peritubular capillaries is:
 - A. Reabsorption
 - B. Filtration
 - C. Secretion
 - D. Both A and C

- **3.** Blood flows through the nephron in the following order:
 - A. Efferent arteriole, peritubular capillaries, vasa recta, afferent arteriole
 - B. Peritubular capillaries, afferent arteriole, vasa recta, efferent arteriole
 - C. Afferent arteriole, peritubular capillaries, vasa recta, efferent arteriole
 - D. Efferent arteriole, vasa recta, peritubular capillaries, afferent arteriole
- **4.** Filtration of protein is prevented in the glomerulus by:
 - A. Hydrostatic pressure
 - B. Oncotic pressure
 - C. Renin
 - D. Capillary pores
- **5.** Renin is secreted by the nephron in response to:
 - A. Low systemic blood pressure
 - B. High systemic blood pressure
 - C. Oncotic capillary pressure
 - D. Increased water retention
- **6.** The primary chemical affected by the reninangiotensin-aldosterone system is:
 - A. Chloride
 - B. Sodium
 - C. Potassium
 - D. Hydrogen
- 7. Secretion of renin is stimulated by:
 - A. Juxtaglomerular cells
 - B. Angiotensin I and II
 - C. Macula densa cells
 - D. Circulating angiotensin-converting enzyme
- **8.** The hormone aldosterone is responsible for:
 - A. Hydrogen ion secretion
 - B. Potassium secretion
 - C. Chloride retention
 - D. Sodium retention
- **9.** The fluid leaving the glomerulus has a specific gravity of:
 - A. 1.005
 - B. 1.010
 - C. 1.015
 - D. 1.020
- **10**. All of the following are reabsorbed by active transport in the tubules *except*:
 - A. Urea
 - B. Glucose
 - C. Sodium
 - D. Chloride

Continued

- 11. Which of the tubules is impermeable to water?
 - A. Proximal convoluted tubule
 - B. Descending loop of Henle
 - C. Ascending loop of Henle
 - D. Distal convoluted tubule
- 12. Glucose will appear in the urine when the:
 - A. Blood level of glucose is 200 mg/dL
 - B. Tm for glucose is reached
 - C. Renal threshold for glucose is exceeded
 - D. All of the above
- 13. The countercurrent mechanism takes place in the:
 - A. Juxtaglomerular nephrons
 - B. Proximal convoluted tubule
 - C. Cortical nephrons
 - D. Both A and C
- **14.** ADH regulates the final urine concentration by controlling:
 - A. Active reabsorption of sodium
 - B. Tubular permeability
 - C. Passive reabsorption of urea
 - D. Passive reabsorption of chloride
- 15. When the body is dehydrated:
 - A. ADH production is decreased
 - B. ADH production is increased
 - C. Urine volume is increased
 - D. Both A and C.
- **16**. Bicarbonate ions filtered by the glomerulus are returned to the blood:
 - A. In the proximal convoluted tubule
 - B. Combined with hydrogen ions
 - C. By tubular secretion
 - D. All of the above
- **17**. If ammonia is not produced by the distal convoluted tubule, the urine pH will be:
 - A. Acidic
 - B. Basic
- **18.** Place the appropriate letter in front of the following clearance substances:
 - A. Exogenous
 - B. Endogenous
 - ____ inulin
 - ____ creatinine
 - ____ cystatin C
 - ____ ¹²⁵I-iothalmate
- **19**. The largest source of error in creatinine clearance tests is:
 - A. Secretion of creatinine
 - B. Improperly timed urine specimens
 - C. Refrigeration of the urine
 - D. Time of collecting blood sample

- **20.** Given the following information, calculate the creatinine clearance:
 - 24-hour urine volume: 1000 mL; serum creatinine:
 - 2.0 mg/dL; urine creatinine: 200 mg/dL
- **21.** Values for creatinine clearance tests on children are corrected for:
 - A. Body size
 - B. Urine volume
 - C. Activity level
 - D. Diet
- **22.** Given the data serum creatinine: 1.1 mg/dL; age: 50 years, and weight: 72 kg, the estimated creatinine clearance using the Cockcroft-Gault formula is:
 - A. 46
 - B. 62
 - C. 82
 - D. 127
- **23.** Variables that may be included in estimated creatinine clearance calculations include all of the following *except*:
 - A. Serum creatinine
 - B. Urine creatinine
 - C. Age
 - D. Blood urea nitrogen
- **24.** An advantage to using cystatin C to monitor GFR is:
 - A. It does not require urine collection
 - B. It is not secreted by the tubules
 - C. It can be measured by immunoassay
 - D. All of the above
- **25**. Solute dissolved in solvent will:
 - A. Decrease vapor pressure
 - B. Lower the boiling point
 - C. Decrease the osmotic pressure
 - D. Lower the specific gravity
- **26**. Substances that may interfere with measurement of urine and serum osmolarity include all of the following *except*:
 - A. Ethanol
 - B. Lactic acid
 - C. Sodium
 - D. Lipids
- 27. The normal serum osmolarity is:
 - A. 50–100 mOsm
 - B. 275-300 mOsm
 - C. 400-500 mOsm
 - D. 3 times the urine osmolarity
- **28.** After controlled fluid intake, the urine-to-serum osmolarity ratio should be at least:
 - A. 1:1
 - B. 2:1
 - C. 3:1
 - D. 4:1

- **29**. Calculate the free water clearance from the following results:
 - urine volume in 6 hours: 720 mL; urine osmolarity: 225 mOsm; plasma osmolarity: 300 mOsm
- **30**. To provide an accurate measure of renal blood flow, a test substance should be completely:
 - A. Filtered by the glomerulus
 - B. Reabsorbed by the tubules
 - C. Secreted when it reaches the distal convoluted tubule
 - D. Cleared on each contact with functional renal tissue
- **31.** Given the following data, calculate the effective renal plasma flow:
 - urine volume in 2 hours: 240 mL; urine PAH: 150 mg/dL; plasma PAH: 0.5 mg/dL
- 32. Renal tubular acidosis can be caused by the:
 - A. Production of excessively acidic urine due to increased filtration of hydrogen ions
 - B. Production of excessively acidic urine due to increased secretion of hydrogen ions
 - C. Inability to produce an acidic urine due to impaired production of ammonia
 - D. Inability to produce an acidic urine due to increased production of ammonia
- **33.** Tests performed to detect renal tubular acidosis after administering an ammonium chloride load include all of the following *except*:
 - A. Urine ammonia
 - B. Arterial pH
 - C. Urine pH
 - D. Titratable acicity

Case Studies and Clinical Situations

- 1. A 44-year-old man diagnosed with acute tubular necrosis has a blood urea nitrogen of 60 mg/dL and a blood glucose level of 100 mg/dL. A 2+ urine glucose is also reported.
 - a. State the renal threshold for glucose.
 - b. What is the significance of the positive urine glucose and normal blood glucose?
- 2. A patient develops a sudden drop in blood pressure.
 - a. Diagram the reactions that take place to ensure adequate blood pressure within the nephrons.
 - b. How do these reactions increase blood volume?
 - c. When blood pressure returns to normal, how does the kidney respond?

- **3.** A physician would like to prescribe a nephrotoxic antibiotic for a 60-year-old man weighing 80 kg. The patient has a serum creatinine level of 1.0 mg/dL.
 - a. How can the physician determine whether it is safe to prescribe this medication before the patient leaves the office?
 - b. Can the medication be prescribed to this patient with a reasonable assurance of safety?
 - c. A creatinine clearance was also run on the patient with the following results: serum creatinine,
 0.9 mg/dL; urine creatinine, 190 mg/dL; 24-hour urine volume, 720 mL. Should the patient continue to take the medication? Justify your answer.
- 4. A laboratory is obtaining erratic serum osmolarity results on a patient who is being monitored at 6 a.m., 12 p.m., 6 p.m., and 12 a.m. Osmolarities are not performed on the night shift; therefore, the midnight specimen is run at the same time as the 6 a.m. specimen.
 - a. What two reasons could account for these discrepancies?
 - b. If the laboratory is using a freezing point osmometer, would these discrepancies still occur? Why or why not?
 - c. If a friend was secretly bringing the patient a pint of whiskey every night, would this affect the results? Explain your answer.
- 5. Following overnight (6 p.m. to 8 a.m.) fluid deprivation, the urine-to-serum osmolarity ratio in a patient who is exhibiting polyuria and polydipsia is 1:1. The ratio remains the same when a second specimen is tested at 10 a.m. Vasopressin is then administered subcutaneously to the patient, and the fluid deprivation is continued until 2 p.m., when another specimen is tested.
 - a. What disorder do these symptoms and initial laboratory results indicate?
 - b. If the urine-to-serum osmolarity ratio on the 2 p.m. specimen is 3:1, what is the underlying cause of the patient's disorder?
 - c. If the urine-to-serum osmolarity ratio on the 2 p.m. specimen remains 1:1, what is the underlying cause of the patient's disorder?













Introduction to Urinalysis

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

- 1 List three major organic and three major inorganic chemical constituents of urine.
- **2** Describe a method for determining whether a questionable fluid is urine.
- 3 Recognize normal and abnormal daily urine volumes.
- **4** Describe the characteristics of the recommended urine specimen containers.
- 5 Describe the correct methodology for labeling urine specimens.
- **6** State four possible reasons why a laboratory would reject a urine specimen.

- 7 List 10 changes that may take place in a urine specimen that remains at room temperature for more than 2 hours.
- **8** Discuss the actions of bacteria on an unpreserved urine specimen.
- 9 Briefly discuss five methods for preserving urine specimens, including their advantages and disadvantages.
- 10 Instruct a patient in the correct procedure for collecting a timed urine specimen and a midstream clean-catch specimen.
- 11 Describe the type of specimen needed for optimal results when a specific urinalysis procedure is requested.

KEY TERMS

anuria
catheterized specimen
chain of custody
fasting specimen
first morning specimen

2-hour postprandial specimen midstream clean-catch specimen nocturia oliguria

polyuria suprapubic aspiration three-glass collection timed specimen

■■● History and Importance

Analyzing urine was actually the beginning of laboratory medicine. References to the study of urine can be found in the drawings of cavemen and in Egyptian hieroglyphics, such as the Edwin Smith Surgical Papyrus. Pictures of early physicians commonly showed them examining a bladder-shaped flask of urine (Fig. 3-1). Often these physicians never saw the patient, only the patient's urine. Although these physi-

cians lacked the sophisticated testing mechanisms now available, they were able to obtain diagnostic information from such basic observations as color, turbidity, odor, volume, viscosity, and even sweetness (by noting that certain specimens attracted ants). These same urine characteristics are still reported by laboratory personnel. However, modern urinalysis has expanded beyond physical examination of urine to include chemical analysis and microscopic examination of urinary sediment.



Figure 3–I Physician examines urine flask. (Courtesy of National Library of Medicine)

Many well-known names in the history of medicine are associated with the study of urine, including Hippocrates, who in the 5th century BC wrote a book on "uroscopy." During the Middle Ages, physicians concentrated their efforts very intensively on the art of uroscopy, receiving instruction in urine examination as part of their training (Fig. 3-2). By 1140 AD, color charts had been developed that described the significance of 20 different colors (Fig. 3-3). Chemical testing progressed from "ant testing" and "taste testing" for glucose to Frederik Dekkers' discovery in 1694 of *albuminuria* by boiling urine.¹

The credibility of urinalysis became compromised when charlatans without medical credentials began offering their predictions to the public for a healthy fee. These charlatans, called "pisse prophets," became the subject of a book published by Thomas Bryant in 1627. The revelations in this book inspired the passing of the first medical licensure laws in England—another contribution of urinalysis to the field of medicine.

The invention of the microscope in the 17th century led to the examination of urinary sediment and to the development by Thomas Addis of methods for quantitating the microscopic sediment. Richard Bright introduced the concept of urinalysis as part of a doctor's routine patient examination in 1827. By the 1930s, however, the number and complexity of the tests performed in a urinalysis had reached a point of impracticality, and urinalysis began to disappear from routine examinations. Fortunately, devel-



Figure 3–2 Instruction in urine examination. (Courtesy of National Library of Medicine)

opment of modern testing techniques rescued routine urinalysis, which has remained an integral part of the patient examination.

Two unique characteristics of a urine specimen account for this continued popularity:

- 1. Urine is a readily available and easily collected specimen.
- 2. Urine contains information, which can be obtained by inexpensive laboratory tests, about many of the body's major metabolic functions.

These characteristics fit in well with the current trends toward preventive medicine and lower medical costs. In fact, the Clinical and Laboratory Standards Institute (CLSI) (formerly NCCLS) defines urinalysis as "the testing of urine with procedures commonly performed in an expeditious, reliable, accurate, safe, and cost-effective manner." Reasons for performing urinalysis identified by CLSI include aiding in the diagnosis of disease, screening asymptomatic populations for undetected disorders, and monitoring the progress of disease and the effectiveness of therapy.²

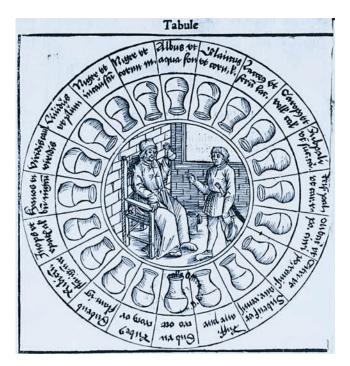


Figure 3–3 A chart used for urine analysis. (Courtesy of National Library of Medicine)

■■● Urine Formation

As detailed in Chapter 2, the kidneys continuously form urine as an ultrafiltrate of plasma. Reabsorption of water and filtered substances essential to body function converts approximately 170,000 mL of filtered plasma to the average daily urine output of 1200 mL.

Urine Composition

In general, urine consists of urea and other organic and inorganic chemicals dissolved in water. Urine is normally 95% water and 5% solutes, although considerable variations in the concentrations of these solutes can occur owing to the influence of factors such as dietary intake, physical activity, body metabolism, endocrine functions, and even body position. Urea, a metabolic waste product produced in the liver from the breakdown of protein and amino acids, accounts for nearly half of the total dissolved solids in urine. Other organic substances include primarily creatinine and uric acid. The major inorganic solid dissolved in urine is chloride, followed by sodium and potassium. Small or trace amounts of many additional inorganic chemicals are also present in urine (Table 3-1). Dietary intake greatly influences the concentrations of these inorganic compounds, making it difficult to establish normal levels. Other substances found in urine include hormones, vitamins, and medications. Although not a part of the original plasma filtrate, the urine also may contain formed elements, such as cells, casts, crystals, mucus, and bacteria. Increased amounts of these formed elements are often indicative of disease.

Should it be necessary to determine whether a particular fluid is urine, the specimen can be tested for its urea and creatinine content. Because both these substances are present in much higher concentrations in urine than in other body fluids, a high urea and creatinine content can identify a fluid as urine.

Urine Volume

Urine volume depends on the amount of water that the kidneys excrete. Water is a major body constituent; therefore, the amount excreted is usually determined by the body's state of hydration. Factors that influence urine volume include fluid intake, fluid loss from nonrenal sources, variations in the secretion of antidiuretic hormone, and need to excrete increased amounts of dissolved solids, such as glucose or salts. Taking these factors into consideration, although the normal daily urine output is usually 1200 to 1500 mL, a range of 600 to 2000 mL is considered normal.

Oliguria, a decrease in urine output, which is less than 1 mL/kg/hr in infants, less than 0.5 mL/kg/hr in children, and less than 400 mL/day in adults, is commonly seen when the body enters a state of dehydration as a result of excessive water loss from vomiting, diarrhea, perspiration, or severe burns. Oliguria leading to anuria, cessation of urine flow, may result from any serious damage to the kidneys or from a decrease in the flow of blood to the kidneys. The kidneys excrete two to three times more urine during the day than during the night. An increase in the nocturnal excretion of urine is termed nocturia. Polyuria, an increase in daily urine volume (greater than 2.5 L/day in adults and 2.5-3 mL/kg/day in children), is often associated with diabetes mellitus and diabetes insipidus; however, it may be artificially induced by diuretics, caffeine, or alcohol, all of which suppress the secretion of antidiuretic hormone.

Diabetes mellitus and diabetes insipidus produce polyuria for different reasons, and analysis of the urine is an important step in the differential diagnosis (Fig. 3-4). Diabetes mellitus is caused by a defect either in the pancreatic production of insulin or in the function of insulin, which results in an increased body glucose concentration. The kidneys do not reabsorb excess glucose, necessitating excretion of increased amounts of water to remove the dissolved glucose from the body. Although appearing to be dilute, a urine specimen from a patient with diabetes mellitus has a high specific gravity because of the increased glucose content.

Diabetes insipidus results from a decrease in the production or function of antidiuretic hormone; thus, the water necessary for adequate body hydration is not reabsorbed from the plasma filtrate. In this condition, the urine is truly dilute and has a low specific gravity. Fluid loss in both diseases is compensated by increased ingestion of water (*polydipsia*), producing an even greater urine volume. Polyuria accompanied by increased fluid intake is often the first symptom of either disease.

Table 3-1 Composition of Urine Collected for 24 Hours		
Component	Amount	Remark
Organic Urea	25.0–35.0 g	60%–90% of nitrogenous material; derived from the metabolism
Creatinine	1.5 g	of amino acids into ammonia Derived from creatine, nitrogenous substance in muscle tissue
Uric acid	0.4–1.0 g	Common component of kidney stones; derived from catabolism of nucleic acid in food and cell destruction
Hippuric acid	0.7 g	Benzoic acid is eliminated from the body in this form; increases with high-vegetable diets
Other substances	2.9 g	Carbohydrates, pigments, fatty acids, mucin, enzymes, hormones; may be present in small amounts depending on diet and health
Inorganic		
Sodium chloride (NaCl)	15.0 g	Principal salt; varies with intake
Potassium (K ⁺)	3.3 g	Occurs as chloride, sulfate, and phosphate salts
Sulfate (SO ₄ ²⁻)	2.5 g	Derived from amino acids
Phosphate (PO ₄ ³⁻)	2.5 g	Occurs primarily as sodium compounds that serve as buffers in the blood
Ammonium (NH ₄ ⁺)	0.7 g	Derived from protein metabolism and glutamine in kidneys; amount varies depending on blood and tissue fluid acidity
Magnesium (Mg2 ⁺)	0.1 g	Occurs as chloride, sulfate, phosphate salts

Adapted from Tortora, GJ, and Anagnostakos, NP: Principles of Anatomy and Physiology, ed 6, Harper & Row, New York, 1990, p. 51.

Occurs as chloride, sulfate, phosphate salts

Specimen Collection

Calcium (Ca2+)

As discussed in Chapter 1, urine is a biohazardous substance that requires the observance of Standard Precautions. Gloves should be worn at all times when in contact with the specimen. Specimens must be collected in clean, dry, leak-proof containers. Disposable containers are recommended because they eliminate the chance of contamination due to improper washing. These disposable containers are available in a variety of sizes and shapes, including bags with adhesive for the collection of pediatric specimens and large containers for 24-hour specimens. Properly applied screw-top lids are less likely to leak than snap-on lids.

0.3 g

Containers for routine urinalysis should have a wide mouth to facilitate collections from female patients and a wide, flat bottom to prevent overturning. They should be made of a clear material to allow for determination of color and clarity. The recommended capacity of the container is 50 mL, which allows 12 mL of specimen needed for microscopic analysis, additional specimen for repeat analysis, and enough room for the specimen to be mixed by swirling the container.

Individually packaged sterile containers with secure closures should be used for microbiologic urine studies. Sterile

containers are also suggested if more than 2 hours elapse between specimen collection and analysis.²

All specimens must be labeled properly with the patient's name and identification number, the date and time of collection, and additional information such as the patient's age and location and the physician's name, as required by institutional protocol. Labels must be attached to the container, not to the lid, and should not become detached if the container is refrigerated or frozen.

A requisition form (manual or computerized) must accompany specimens delivered to the laboratory. The information on the form must match the information on the specimen label. Additional information on the form can include method of collection or type of specimen, possible interfering medications, and the patient's clinical information. The time the specimen is received in the laboratory should be recorded on the form.

Improperly labeled and collected specimens should be rejected by the laboratory, and appropriate personnel should be notified to collect a new specimen. Unacceptable situations include specimens in unlabeled containers, nonmatching labels and requisition forms, specimens contaminated with feces or toilet paper, containers with contaminated exteriors, specimens of insufficient quantity, and specimens that have

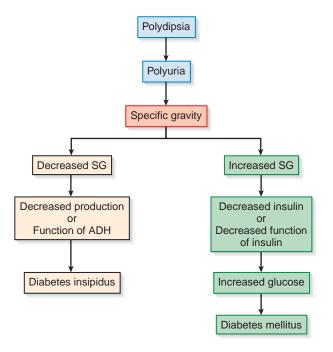


Figure 3–4 Differentiation between diabetes mellitus and diabetes insipidus.

been improperly transported. Laboratories should have a written policy detailing their conditions for specimen rejection (see Chapter 7).

Specimen Handling

The fact that a urine specimen is so readily available and easily collected often leads to laxity in the treatment of the specimen after its collection. Changes in urine composition take

place not only in vivo but also in vitro, thus requiring correct handling procedures.

Specimen Integrity

Following collection, specimens should be delivered to the laboratory promptly and tested within 2 hours. A specimen that cannot be delivered and tested within 2 hours should be refrigerated or have an appropriate chemical preservative added. Table 3–2 describes the 11 most significant changes that may occur in a specimen allowed to remain unpreserved at room temperature for longer than 2 hours. Notice that most of the changes are related to the presence and growth of bacteria.

These variations are discussed again under the individual test procedures. At this point it is important to realize that improper preservation can seriously affect the results of a routine urinalysis.

Specimen Preservation

The most routinely used method of preservation is refrigeration at 2°C to 8°C, which decreases bacterial growth and metabolism. If the urine is to be cultured, it should be refrigerated during transit and held refrigerated until cultured up to 24 hours.² Refrigeration can increase the specific gravity, when measured by urinometer, and the precipitation of amorphous phosphates and urates, which may obscure the microscopic sediment analysis. The specimen must return to room temperature before chemical testing by reagent strips. This will correct the specific gravity and may dissolve some of the amorphous urates.

When a specimen must be transported over a long distance and refrigeration is impossible, chemical preservatives

Table 3-2 Changes in Unpreserved Urine		
Analyte	Change	Cause
Color	Modified/darkened	Oxidation or reduction of metabolites
Clarity	Decreased	Bacterial growth and precipitation of amorphous material
Odor	Increased	Bacterial multiplication or breakdown of urea to ammonia
рН	Increased	Breakdown of urea to ammonia by urease-producing bacteria/ loss of CO_2
Glucose	Decreased	Glycolysis and bacterial use
Ketones	Decreased	Volatilization and bacterial metabolism
Bilirubin	Decreased	Exposure to light/photo oxidation to biliverdin
Urobilinogen	Decreased	Oxidation to urobilin
Nitrite	Increased	Multiplication of nitrate-reducing bacteria
Red and white blood cells and casts	Decreased	Disintegration in dilute alkaline urine
Bacteria	Increased	Multiplication

34 CHAPTER 3 • Introduction to Urinalysis

may be added. Commercially prepared transport tubes are available. The ideal preservative should be bactericidal, inhibit urease, and preserve formed elements in the sediment. At the same time, the preservative should not interfere with chemical tests. Unfortunately, as can be seen in Table 3–3, the ideal preservative does not exist; therefore, a preservative that best suits the needs of the required analysis should be chosen.

■■● Types of Specimens

To obtain a specimen that is representative of a patient's metabolic state, regulation of certain aspects of specimen collection is often necessary. These special conditions may include time, length, and method of collection and the patient's dietary and medicinal intake. It is important to instruct patients when they must follow special collection procedures. Frequently encountered specimens are listed in Table 3–4.

Random Specimen

This is the most commonly received specimen because of its ease of collection and convenience for the patient. The *random specimen* may be collected at any time, but the actual

time of voiding should be recorded on the container.² The random specimen is useful for routine screening tests to detect obvious abnormalities. However, it may also show erroneous results resulting from dietary intake or physical activity just before collection. The patient will then be requested to collect additional specimens under more controlled conditions.

First Morning Specimen

Although it may require the patient to make an additional trip to the laboratory, this is the ideal screening specimen. It is also essential for preventing false-negative pregnancy tests and for evaluating orthostatic *proteinuria*. The *first morning specimen*, or 8-hour specimen, is a concentrated specimen, thereby assuring detection of chemicals and formed elements that may not be present in a dilute random specimen. The patient should be instructed to collect the specimen immediately on arising and to deliver it to the laboratory within 2 hours.

Fasting Specimen (Second Morning)

A *fasting specimen* differs from a first morning specimen by being the second voided specimen after a period of fasting.

Table 3-3 Urine Preservatives			
Preservatives	Advantages	Disadvantages	Additional Information
Refrigeration	Does not interfere with chemical tests	Raises specific gravity by hydrometer Precipitates amorphous phosphates and urates	Prevents bacterial growth 24 h ³
Thymol	Preserves glucose and sediments well	Interferes with acid precipitation tests for protein	
Boric acid	Preserves protein and formed elements well	May precipitate crystals when used in large amounts	Keeps pH at about 6.0
	Does not interfere with routine analyses other than pH		Is bacteriostatic (not bactericidal) at 18 g/L; can use for culture transport ⁴ Interferes with drug and hormone analyses
Formalin (formaldehyde)	Excellent sediment preservative	Acts as a reducing agent, interfering with chemical tests for glucose, blood, leukocyte esterase, and copper reduction	Rinse specimen container with formalin to preserve cells and casts
Toluene	Does not interfere with routine tests	Floats on surface of speci- mens and clings to pipettes and testing materials	
Sodium fluoride	Prevents glycolysis	Inhibits reagent strip tests for glucose, blood, and leukocytes	May use sodium benzoate instead of fluoride for reagent strip testing ⁵
	Is a good preservative for drug analyses		

Preservatives	Advantages	Disadvantages	Additional Information
Phenol	Does not interfere with routine tests	Causes an odor change	Use 1 drop per ounce of specimen
Commercial preservative tablets	Convenient when refrigera- tion not possible Have controlled concentra- tion to minimize interference	May contain one or more of the preservatives including sodium fluoride	Check tablet composition to determine possible effects on desired tests
Urine Collection Kits ⁶ (Becton Dickinson, Rutherford, NJ)	Contains collection cup, C&S preservative tube or UA tube		
Gray C&S tube	Sample stable at room tem- perature (RT) for 48 hr; preserves bacteria	Decreases pH; do not use if urine is below minimum fill line	Preservative is boric acid and may not be used for UA
Yellow plain UA tube	Use on automated instruments	Must refrigerate within 2 hours	Round or conical bottom
Cherry red/yellow top tube	Stable for 72 hours at RT; instrument-compatible	Bilirubin and urobilinogen may be decreased if specimen is exposed to light and left at RT	Preservative is sodium propionate; conical bottom
Saccomanno Fixative	Preserves cellular elements		Used for cytology studies

Table 3-4 Types of Urine Specimens		
Type of Specimen	Purpose	
Random	Routine screening	
First morning	Routine screening Pregnancy tests Orthostatic protein	
Fasting (second morning)	Diabetic screening/monitoring	
2-hour postprandial	Diabetic monitoring	
Glucose tolerance test	Optional with blood samples in glucose tolerance test	
24-h (or timed)	Quantitative chemical tests	
Catheterized	Bacterial culture	
Midstream clean-catch	Routine screening Bacterial culture	
Suprapubic aspiration	Bladder urine for bacterial culture Cytology	
Three-glass collection	Prostatic infection	

This specimen will not contain any metabolites from food ingested before the beginning of the fasting period. It is recommended for glucose monitoring.⁷

2-Hour Postprandial Specimen

The patient is instructed to void shortly before consuming a routine meal and to collect a specimen 2 hours after eating. The specimen is tested for glucose, and the results are used primarily for monitoring insulin therapy in persons with diabetes mellitus. A more comprehensive evaluation of the patient's status can be obtained if the results of the **2-hour postprandial specimen** are compared with those of a fasting specimen and corresponding blood glucose tests.

Glucose Tolerance Specimens

Glucose tolerance specimens are sometimes collected to correspond with the blood samples drawn during a glucose tolerance test (GTT). The number of specimens varies with the length of the test. GTTs may include fasting, half-hour, 1-hour, 2-hour, and 3-hour specimens, and possibly 4-hour, 5-hour, and 6-hour specimens. The urine is tested for glucose and ketones, and the results are reported along with the blood test results as an aid to interpreting the patient's ability to metabolize a measured amount of glucose and are correlated with the renal threshold for glucose. Collection of these specimens is an institutional option.⁸

PROCEDURE



24-Hour (Timed) Specimen Collection Procedure

Provide patient with written instructions, and explain collection procedure.

Issue proper collection container and preservative.

Day 1: 7 a.m.: patient voids and discards specimen; collects all urine for the next 24 hours.

Day 2: 7 a.m.: patient voids and adds this urine to previously collected urine.

On arrival at laboratory, the entire 24-hour specimen is thoroughly mixed, and the volume is measured and recorded.

An aliquot is saved for testing and additional or repeat testing; discard remaining urine.

24-Hour (or Timed) Specimen

Measuring the exact amount of a urine chemical is often necessary instead of just reporting its presence or absence. A carefully *timed specimen* must be used to produce accurate quantitative results. Many solutes exhibit diurnal variations such as catecholamines, 17-hydroxysteroids, and electrolytes in which the lowest concentration is in the early morning and the highest concentration occurs in the afternoon.² When the concentration of the substance to be measured changes with diurnal variations and with daily activities such as exercise, meals, and body metabolism, 24-hour collection is required. If the concentration of a particular substance remains constant, the specimen may be collected over a shorter period. Care must be taken, however, to keep the patient adequately hydrated during short collection periods. Patients must be instructed on the procedure for collecting a timed specimen.

To obtain an accurate timed specimen, the patient must begin and end the collection period with an empty bladder. The concentration of a substance in a particular period must be calculated from the urine volume produced during that time. Addition of urine formed before the start of the collection period or failure to include urine produced at the end of the collection period will produce inaccurate results.

On its arrival in the laboratory, a 24-hour specimen must be thoroughly mixed and the volume accurately measured and recorded. If only an aliquot is needed for testing, the amount saved must be adequate to permit repeat or additional testing. If a specimen is collected in two containers, the contents of the containers should be combined and thoroughly mixed before aliquoting. Consideration also must be given to the preservation of specimens collected over extended periods. All specimens should be refrigerated or kept on ice during the collection period and may also require addition of a chemical preservative. The preservative chosen must be nontoxic to the patient and should not interfere with the tests to be performed. Appropriate collection information

is included with test procedures and should be read before issuing a container and instructions to the patient.

Catheterized Specimen

This specimen is collected under sterile conditions by passing a hollow tube (catheter) through the urethra into the bladder. The most commonly requested test on a *catheterized specimen* is a bacterial culture. If a routine urinalysis is also requested, the culture should be performed first to prevent contamination of the specimen.

A less frequently encountered type of catheterized specimen measures functions in the individual kidneys. Specimens from the right and left kidneys are collected separately by passing catheters through the ureters of the respective kidneys.

Midstream Clean-Catch Specimen

As an alternative to the catheterized specimen, the *midstream* clean-catch specimen provides a safer, less traumatic method for obtaining urine for bacterial culture and routine urinalysis. It provides a specimen that is less contaminated by epithelial cells and bacteria and, therefore, is more representative of the actual urine than the routinely voided specimen. Patients must be provided with appropriate cleansing materials, a sterile container, and instructions for cleansing and voiding. Strong bacterial agents, such as hexachlorophene or povidone-iodine, should not be used as cleansing agents. Mild antiseptic towelettes are recommended. Patients are instructed to wash their hands before beginning the collection. Male patients should clean the glans, which begins at the urethra, and withdraw the foreskin, if necessary. Female patients should separate the labia and clean the urinary meatus and surrounding area. When cleansing is complete, patients are to void first into the toilet, then collect an adequate amount of urine in the sterile container, and finish voiding into the toilet. Care should be taken not to contaminate the specimen container. As with a catheterized specimen, if a routine urinalysis is also requested, the culture should be performed first to prevent contamination of the specimen.

Suprapubic Aspiration

Occasionally urine may be collected by external introduction of a needle through the abdomen into the bladder. Because the bladder is sterile under normal conditions, *suprapubic aspiration* provides a sample for bacterial culture that is completely free of extraneous contamination. The specimen can also be used for cytologic examination.

Prostatitis Specimen

Similar to the midstream clean-catch collection, the *three-glass collection* procedure is used to determine prostatic infection. Instead of discarding the first urine passed, it is collected in a sterile container. Next, the midstream portion is collected in another sterile container. The prostate is then massaged so that prostate fluid will be passed with the

remaining urine into a third sterile container. Quantitative cultures are performed on all specimens, and the first and third specimens are examined microscopically. In prostatic infection, the third specimen will have a white blood cell/high-power field count and a bacterial count 10 times that of the first specimen. Macrophages containing lipids may also be present. The second specimen is used as a control for bladder and kidney infection. If it is positive, the results from the third specimen are invalid because infected urine has contaminated the specimen.9 Variations of the procedure include the Stamey-Mears four-glass localization method and the preand postmassage test (PPMT). The four-glass method consists of bacterial cultures of the initial voided urine (VB1), midstream urine (VB2), expressed prostatic secretions (EPS), and a postprostatic massage urine specimen (VB3). Urethral infection or inflammation is tested for by the VB1, and the VB2 is tested for urinary bladder infection. The prostatic secretions are cultured and examined for white bood cells. More than 10 to 20 white blood cells per high-power field is considered abnormal. In the PPMT test, a clean-catch midstream urine specimen is collected. A second urine sample is collected after the prostate is massaged. A positive result is significant bacteriuria in the postmassage specimen of greater than 10 times the premassage count.10

Pediatric Specimen

Collection of pediatric specimens can present a challenge. Soft, clear plastic bags with hypoallergenic skin adhesive to attach to the genital area of both boys and girls are available for collecting routine specimens. Sterile specimens may be obtained by catheterization or by suprapubic aspiration. Specimens for culture also may be obtained using a clean-catch cleansing procedure and a sterile collection bag. Care must be taken not to touch the inside of the bag when applying it. For quantitative testing, bags are available that allow a tube to be attached and excess urine transferred to a larger container.

Drug Specimen Collection

Urine specimen collection is the most vulnerable part of a drug-testing program. Correct collection procedures and documentation are necessary to ensure that the results are those of the specific individual submitting the specimen. The *chain of custody* (COC) is the process that provides this documentation of proper sample identification from the time of collection to the receipt of laboratory results. The COC is a standardized form that must document and accompany every step of drug testing, from collector to courier to laboratory to medical review officer to employer.

For urine specimens to withstand legal scrutiny, it is necessary to prove that no tampering of the specimen occurred, such as substitution, adulteration, or dilution. All personnel handling the specimen must be noted. The specimen must be handled securely, with a guarantee that no unauthorized access to the specimen was possible. Proper identification of the individual whose information is indicated on the label is

PROCEDURE



Urine Drug Specimen Collection Procedure^{11,12}

- 1. The collector washes hands and wears gloves.
- **2.** The collector adds bluing agent (dye) to the toilet water reservoir to prevent an adulterated specimen.
- The collector eliminates any source of water other than toilet by taping the toilet lid and faucet handles.
- **4.** The donor provides photo identification or positive identification from employer representative.
- The collector completes step 1 of the chain of custody (COC) form and has the donor sign the form.
- 6. The donor leaves his or her coat, briefcase, and/or purse outside the collection area to avoid the possibility of concealed substances contaminating the urine.
- 7. The donor washes his or her hands and receives a specimen cup.
- **8**. The collector remains in the restroom but outside the stall, listening for unauthorized water use, unless a witnessed collection is requested.
- The donor hands specimen cup to the collector. Transfer is documented.
- **10**. The collector checks the urine for abnormal color and for the required amount (30–45 mL).
- 11. The collector checks that the temperature strip on the specimen cup reads 32.5°–37.7°C. The collector records the in-range temperature on the COC form (COC step 2). If the specimen temperature is out of range or the specimen is suspected to have been diluted or adulterated, a new specimen must be collected and a supervisor notified.
- **12**. The specimen must remain in the sight of the donor and collector at all times.
- 13. With the donor watching, the collector peels off the specimen identification strips from the COC form (COC step 3) and puts them on the capped bottle, covering both sides of the cap.
- 14. The donor initials the specimen bottle seals.
- 15. The date and time are written on the seals.
- 16. The donor completes step 4 on the COC form.
- 17. The collector completes step 5 on the COC form.
- 18. Each time the specimen is handled, transferred, or placed in storage, every individual must be identified and the date and purpose of the change recorded.
- 19. The collector follows laboratory-specific instructions for packaging the specimen bottles and laboratory copies of the COC form.
- **20**. The collector distributes the COC copies to appropriate personnel.

required. Either photo identification or positive identification by an employer representative with photo ID is acceptable.

Urine specimen collections may be "witnessed" or "unwitnessed." The decision to obtain a witnessed collection is indicated when it is suspected that the donor may alter or substitute the specimen or it is the policy of the client ordering the test. If a witnessed specimen collection is ordered, a same-gender collector will observe the collection of 30 to 45 mL of urine. Witnessed and unwitnessed collections should be immediately handed to the collector.

The urine temperature must be taken within 4 minutes from the time of collection to confirm the specimen has not been adulterated. The temperature should read within the range of 32.5°C to 37.7°C. If the specimen temperature is not within range, the temperature should be recorded and the supervisor or employer contacted immediately. Urine temperatures outside of the recommended range may indicate specimen contamination. Recollection of a second specimen as soon as possible will be necessary. The urine color is inspected to identify any signs of contaminants. The specimen is labeled, packaged, and transported following laboratory-specific instructions.

References

- 1. Herman, JR: Urology: A View Through the Retrospectroscope. Harper & Row, Hagerstown, Md., 1973.
- Clinical and Laboratory Standards Institute (formerly NCCLS), Approved Guideline GP16-A2: Urinalysis and Collection, Transportation, and Preservation of Urine Specimens; Approved Guideline—Second Edition, CLSI, formerly NCCLS, Wayne, Pa. 2001
- 3. Culhane, JK: Delayed analysis of urine. J Fam Pract 30(4): 473-474, 1990.
- 4. Meers, PD, and Chow, CK: Bacteriostatic and bactericidal actions of boric acid against bacteria and fungi commonly found in urine. J Clin Pathol 43:484-487, 1990.
- Onstad, J, Hancock, D, and Wolf, P: Inhibitory effect of fluoride on glucose tests with glucose oxidase strips. Clin Chem 21:898-899, 1975.
- Becton, Dickinson and Company: BD Vacutainer® Urine Products for collection, storage, and transport of urine specimens. Product Circular, 2004.
- 7. Guthrie, D, Hinnen, D, and Guthrie, R: Single-voided vs. double-voided urine testing. Diabetes Care 2(3):269-271, 1979
- 8. Baer, DM: Glucose tolerance test: Tips from the clinical experts. Medical Laboratory Observer, Sept. 2003.
- 9. Rous, SN: The Prostate Book. Consumers Union, Mt. Vernon, N.Y., 1988.
- 10. Stevermer, JJ, and Easley, SK: Treatment of prostatitis. Am Fam Physician 61(10), 2000.
- Saint Joseph Hospital Toxicology Laboratory/Creighton Medical Laboratories, Urine Drug Screening Collection Procedure, Omaha, Nebr., 1996.
- STA United, Inc. and the Nebraska Department of Roads Federal Transit Administration Compliance 101 Seminar Workbook. Omaha, Nebr., 1996.

QUESTIONS

- **1.** The primary chemical constituents of normal urine are:
 - A. Protein, sodium, and water
 - B. Urea, water, and protein
 - C. Urea, chloride, and water
 - D. Urea, bilirubin, and glucose
- **2.** An unidentified fluid is received in the laboratory with a request to determine if the fluid is urine or another body fluid. Using routine laboratory tests, what tests would determine that the fluid is most probably urine?
 - A. Glucose and ketones
 - B. Urea and creatinine
 - C. Uric acid and amino acids
 - D. Protein and amino acids
- **3.** A person exhibiting oliguria would have a daily urine volume of:
 - A. 200-400 mL
 - B. 600-1000 mL
 - C. 1000-1500 mL
 - D. Over 1500 mL
- **4.** A patient presenting with polyuria, nocturia, polydipsia, and a high urine specific gravity is exhibiting symptoms of what disorder?
 - A. Diabetes insipidus
 - B. Diabetes mellitus
 - C. Urinary tract infection
 - D. Uremia
- **5.** *True or False:* Disposable containers with a capacity of 50 mL are recommended for the collection of specimens for routine urinalysis.
- **6.** The correct method for labeling urine specimen containers is to:
 - A. Attach the label to the lid
 - B. Attach the label to the bottom
 - C. Attach the label to the container
 - D. Use only a wax pencil for labeling
- 7. A urine specimen for routine urinalysis would be rejected by the laboratory because:
 - A. The specimen had been refrigerated
 - B. More than 50 mL was in the container
 - C. The specimen and accompanying requisition did not match
 - D. The label was placed on the side of the container

- **8.** An unpreserved specimen collected at 8 a.m. and remaining at room temperature until the afternoon shift arrives can be expected to have:
 - 1. Decreased glucose and ketones
 - 2. Increased bacteria and nitrite
 - 3. Decreased pH and turbidity
 - 4. Increased cellular elements
 - A. 1, 2, and 3
 - B. 1, 2, and 4
 - C. 1 and 2 only
 - D. 4 only
- **9.** A specimen containing precipitated amorphous urates may have been preserved using:
 - A. Boric acid
 - B. Chloroform
 - C. Formalin
 - D. Refrigeration
- **10.** What three changes will affect the results of the microscopic examination of urine if it is not tested within 2 hours?
 - A. Decreased bacteria, decreased red blood cells, decreased casts
 - B. Increased bacteria, increased red blood cells, increased casts
 - C. Increased bacteria, decreased red blood cells, decreased casts
 - D. Decreased bacteria, increased red blood cells, increased casts
- **11.** What is the method of choice for preservation of routine urinalysis samples?
 - A. Boric acid
 - B. Formalin
 - C. Refrigeration
 - D. Sodium fluoride
- **12**. For best preservation of urinary sediments, the preservatives of choice are:
 - A. Boric acid and thymol
 - B. Formalin and sodium fluoride
 - C. Toluene and freezing
 - D. Chloroform and refrigeration
- **13**. What chemical can be used to preserve a specimen for a culture and a routine urinalysis?
 - A. Boric acid
 - B. Formalin
 - C. Sodium fluoride
 - D. Thymol

- **14.** *True or False*: A properly labeled urine specimen for routine urinalysis delivered to the laboratory in a gray-top blood collection tube can be tested.
- 15. What is the specimen of choice for routine urinalysis?
 - A. Fasting specimen
 - B. First morning specimen
 - C. Random specimen
 - D. 24-Hour specimen
- 16. Quantitative urine tests are performed on:
 - A. First morning specimens
 - B. Timed specimens
 - C. Midstsream clean-catch specimens
 - D. Suprapubic aspirations
- **17**. Three types of urine specimens that would be acceptable for culture to diagnose a bladder infection include all of the following *except*:
 - A. Catheterized
 - B. Midstream clean-catch
 - C. Random
 - D. Suprapubic aspiration
- **18**. A negative urine pregnancy test performed on a random specimen may need to be repeated using a:
 - A. Clean-catch specimen
 - B. Fasting specimen
 - C. First morning specimen
 - D. 24-Hour specimen
- 19. Cessation of urine flow is termed:
 - A. Anuria
 - B. Azotemia
 - C. Diuresis
 - D. Dysuria
- **20**. Persons taking diuretics can be expected to produce:
 - A. Oliguria
 - B. Polyuria
 - C. Proteinuria
 - D. Pyuria
- **21.** What type of urine specimen should be collected from a patient who complains of painful urination and the physician has ordered a routine urinalysis and urine culture?
 - A. Random
 - B. First morning
 - C. Fasting
 - D. Midstream clean-catch

40 CHAPTER 3 • Introduction to Urinalysis

Case Studies and Clinical Situations

- **1.** A 24-hour urine collection received in the laboratory for creatinine analysis has a volume of 500 mL.
 - a. Should this specimen be rejected and a new specimen requested? Why or why not?
 - b. State a possible source of error, if the creatinine concentration per 24 hours is abnormally low.
- 2. Mary Johnson brings a urine specimen to the laboratory for a glucose analysis. The test result is negative. The physician questions the result because the patient has a family history of diabetes mellitus and is experiencing mild clinical symptoms.
 - a. What two sources of error related to the urine specimen could account for the negative test result?
 - b. How could a specimen be collected that would more accurately reflect Mary's glucose metabolism?
- **3.** A three-glass specimen for determination of possible prostatic infection is sent to the laboratory. Specimens #1 and #3 contain increased white blood cell levels.

- a. If all three specimens have positive bacterial cultures, does the patient have a prostatic infection? Explain your answer.
- b. Why is the presence of white blood cells in specimen #2 not part of the examination?
- c. If the amount of bacteria and white blood cells in specimen #1 is significantly lower than in specimen #3, what is the significance?
- 4. A worker suspects that he or she will be requested to collect an unwitnessed urine specimen for drug analysis. He or she carries a substitute specimen in his or her pocket for 2 days before being told to collect the specimen. Shortly after the worker delivers the specimen, he or she is instructed to collect another specimen.
 - a. What test was performed on the specimen to determine possible specimen manipulation?
 - b. How was the specimen in this situation affected?
 - c. If a specimen for drug analysis tests positive, state a possible defense related to specimen collection and handling that an attorney might employ.
 - d. How can this defense be avoided?











Physical Examination of Urine

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

- 1 List the common terminology used to report normal urine color.
- **2** Discuss the relationship of urochrome to normal urine color.
- **3** State how the presence of bilirubin in a specimen may be suspected.
- **4** Discuss the significance of cloudy red urine and clear red urine.
- 5 Name two pathologic causes of black or brown urine
- **6** Discuss the significance of phenazopyridine in a specimen.
- 7 State the clinical significance of urine clarity.
- **8** List the common terminology used to report clarity.
- **9** Describe the appearance and discuss the significance of amorphous phosphates and amorphous urates in freshly voided urine.

- **10** List three pathologic and four nonpathologic causes of cloudy urine.
- 11 Define specific gravity, and tell why this measurement can be significant in the routine analysis.
- 12 Describe the principles of the urinometer, refractometer, and harmonic oscillation densitometry methods for determining specific gravity.
- **13** State two advantages of performing specific gravity by refractometer rather than by urinometer.
- 14 Given the concentration of glucose and protein in a specimen, calculate the correction needed to compensate for these high-molecular-weight substances in the refractometer specific gravity reading.
- 15 Name two nonpathogenic causes of abnormally high specific gravity readings using a refractioneter
- **16** State possible causes of abnormal urine odor.

KEY TERMS

clarity harmonic oscillation densitometry hypersthenuric hyposthenuric isosthenuric refractometry specific gravity urinometry

42 CHAPTER 4 • Physical Examination of Urine

The physical examination of urine includes the determination of the urine color, *clarity*, and *specific gravity*. As mentioned in Chapter 3, early physicians based many medical decisions on the color and clarity of urine. Today, observation of these characteristics provides preliminary information concerning disorders such as glomerular bleeding, liver disease, inborn errors of metabolism, and urinary tract infection. Measurement of specific gravity aids in the evaluation of renal tubular function. The results of the physical portion of the urinalysis also can be used to confirm or to explain findings in the chemical and microscopic areas of urinalysis.

Color

The color of urine varies from almost colorless to black. These variations may be due to normal metabolic functions, physical activity, ingested materials, or pathologic conditions. A noticeable change in urine color is often the reason a patient seeks medical advice; it then becomes the responsibility of the laboratory to determine whether this color change is normal or pathologic. The more common normal and pathologic correlations of urine colors are summarized in Table 4–1.

Normal Urine Color

Terminology used to describe the color of normal urine may differ slightly among laboratories but should be consistent within each laboratory. Common descriptions include pale yellow, yellow, dark yellow, and amber. Care should be taken to examine the specimen under a good light source, looking down through the container against a white background. The yellow color of urine is caused by the presence of a pigment, which Thudichum named *urochrome* in 1864. Urochrome is a product of endogenous metabolism, and under normal conditions the body produces it at a constant rate. The actual amount of urochrome produced is dependent on the body's metabolic state, with increased amounts produced in thyroid conditions and fasting states. ¹ Urochrome also increases in urine that stands at room temperature. ²

Because urochrome is excreted at a constant rate, the intensity of the yellow color in a fresh urine specimen can give a rough estimate of urine concentration. A dilute urine will be pale yellow and a concentrated specimen will be dark yellow. Remember that, owing to variations in the body's state of hydration, these differences in the yellow color of urine can be normal.

Table 4-1 Laboratory Correlation of Urine Color ¹¹		n of Urine Color ^{II}
Color	Cause	Clinical/Laboratory Correlations
Colorless	Recent fluid consumption	Commonly observed with random specimens
Pale yellow	Polyuria or diabetes insipidus Diabetes mellitus Dilute random specimen	Increased 24-hour volume Elevated specific gravity and positive glucose test result Recent fluid consumption
Dark yellow	Concentrated specimen	May be normal after strenuous exercise or in first morning specimen
Amber		Deydration from fever or burns
Orange	Bilirubin	Yellow foam when shaken and positive chemical test results for bilirubin
	Acriflavine	Negative bile test results and possible green fluorescence
	Phenazopyridine (Pyridium)	Drug commonly administered for urinary tract infections May have orange foam and thick orange pigment that can obscure or interfere with reagent strip readings
	Nitrofurantoin	Antibiotic administered for urinary tract infections
	Phenindione	Anticoagulant, orange in alkaline urine, colorless in acid urine
Yellow-green Yellow-brown	Bilirubin oxidized to biliverdin	Colored foam in acidic urine and false-negative chemical test results for bilirubin
Green	Pseudomonas infection	Positive urine culture
Blue-green	Amitriptyline Methocarbamol (Robaxin) Clorets Indican Methylene blue Phenol	Antidepressant Muscle relaxant, may be green-brown None Bacterial infections Fistulas When oxidized

Color	Cause	Clinical/Laboratory Correlations
Pink Red	RBCs	Cloudy urine with positive chemical test results for blood and RBCs visible microscopically
	Hemoglobin	Clear urine with positive chemical test results for blood; intravascular hemolysis
	Myoglobin	Clear urine with positive chemical test results for blood; muscle damage
	Porphyrins	Negative chemical test results for blood Detect with Watson-Schwartz screening test or fluorescence under ultra- violet light
	Beets	Alkaline urine of genetically susceptible persons
	Rifampin	Tuberculosis medication
	Menstrual contamination	Cloudy specimen with RBCs, mucus, and clots
Brown Black	RBCs oxidized to methemoglobin	Seen in acidic urine after standing; positive chemical test result for blood
	Methemoglobin	Denatured hemoglobin
	Homogentisic acid (alkaptonuria)	Seen in alkaline urine after standing; specific tests are available
	Melanin or melanogen	Urine darkens on standing and reacts with nitroprusside and ferric chloride
	Phenol derivatives	Interfere with copper reduction tests
	Argyrol (antiseptic)	Color disappears with ferric chloride
	Methyldopa or levodopa	Antihypertensive
	Metronidazole (Flagyl)	Darkens on standing

Two additional pigments, *uroerythrin* and *urobilin*, are also present in the urine in much smaller quantities and contribute little to the color of normal, fresh urine. The presence of uroerythrin, a pink pigment, is most evident in specimens that have been refrigerated, resulting in the precipitation of amorphous urates. Uroerythrin attaches to the urates, producing a pink color to the sediment. Urobilin, an oxidation product of the normal urinary constituent urobilinogen, imparts an orange-brown color to urine that is not fresh.

Abnormal Urine Color

As can be seen in Table 4–1, abnormal urine colors are as numerous as their causes. Certain colors, however, are seen more frequently and have a greater clinical significance than others.

Dark Yellow/Amber/Orange

Dark yellow or amber urine may not always signify a normal concentrated urine but can be caused by the presence of the abnormal pigment bilirubin. If bilirubin is present, it will be detected during the chemical examination; however, its presence is suspected if a yellow foam appears when the specimen is shaken. Normal urine produces only a small amount of rapidly disappearing foam when shaken, and a large amount of white foam indicates an increased concentration of protein. A urine specimen that contains bilirubin may also contain hepatitis virus, reinforcing the need to follow standard precau-

tions. The photo-oxidation of large amounts of excreted urobilinogen to urobilin also produces a yellow-orange urine; however, yellow foam does not appear when the specimen is shaken. Photo-oxidation of bilirubin imparts a yellow-green color to the urine.

Also frequently encountered in the urinalysis laboratory is the yellow-orange specimen caused by the administration of phenazopyridine (Pyridium) or azo-gantrisin compounds to persons with urinary tract infections. This thick, orange pigment not only obscures the natural color of the specimen but also interferes with chemical tests that are based on color reactions. Recognition of the presence of phenazopyridine in a specimen is important so that laboratories can use alternate testing procedures. Specimens containing phenazopyridine produce a yellow foam when shaken, which could be mistaken for bilirubin.

Red/Pink/Brown

One of the most common causes of abnormal urine color is the presence of blood. Red is the usual color that blood produces in urine, but the color may range from pink to brown, depending on the amount of blood, the pH of the urine, and the length of contact. Red blood cells (RBCs) remaining in an acidic urine for several hours produce a brown urine due to the oxidation of hemoglobin to methemoglobin. A fresh brown urine containing blood may also indicate glomerular bleeding resulting from the conversion of hemoglobin to methemoglobin.³

44 CHAPTER 4 • Physical Examination of Urine

Besides RBCs, two other substances, hemoglobin and myoglobin, produce a red urine and result in a positive chemical test result for blood (Fig. 4-1). When RBCs are present, the urine is red and cloudy; however, if hemoglobin or myoglobin is present, the specimen is red and clear. Distinguishing between hemoglobinuria and myoglobinuria may be possible by examining the patient's plasma. Hemoglobinuria resulting from the in vivo breakdown of RBCs is accompanied by red plasma. Breakdown of skeletal muscle produces myoglobin. Myoglobin is more rapidly cleared from the plasma than is hemoglobin and, therefore, does not affect the color of the plasma. Fresh urine containing myoglobin frequently exhibits a more reddish-brown color than hemoglobin. The possibility of hemoglobinuria being produced from the in vitro lysis of RBCs also must be considered. Chemical tests to distinguish between hemoglobin and myoglobin are available (see Chapter 5).

Urine specimens containing porphyrins also may appear red resulting from the oxidation of *porphobilinogen* to *porphyrins*. They are often referred to as having the color of port wine.

Nonpathogenic causes of red urine include menstrual contamination, ingestion of highly pigmented foods, and medications. In genetically susceptible persons, eating fresh beets causes a red color in alkaline urine. Ingestion of blackberries can produce a red color in acidic urine. Many medications, including rifampin, phenolphthalein, phenindione, and phenothiazines, produce red urine.

Brown/Black

Additional testing is recommended for urine specimens that turn brown or black on standing and have negative chemical test results for blood, inasmuch as they may contain melanin or homogentisic acid. Melanin is an oxidation product of the colorless pigment, melanogen, produced in excess when a malignant *melanoma* is present. Homogentisic acid, a metabolite of phenylalanine, imparts a black color to alkaline urine from persons with the inborn-error of metabolism, called alkaptonuria. These conditions are discussed in Chapter 9. Medications producing brown/black urines include levodopa, methyldopa, phenol derivatives, and metronidazole (Flagyl).

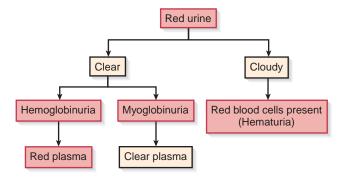


Figure 4–I Differentiation of red urine testing chemically positive for blood.

Blue/Green

Pathogenic causes of blue/green urine color are limited to bacterial infections, including urinary tract infection by *Pseudomonas* species and intestinal tract infections resulting in increased urinary indican. Ingestion of breath deodorizers (Clorets) can result in a green urine color. The medications methocarbamol (Robaxin), methylene blue, and amitriptyline (Elavil) may cause blue urine.

Observation of specimen collection bags from hospitalized patients frequently detects abnormally colored urine. This may signify a pathologic condition that requires the urine to stand for a period of time before color development or the presence of medications. Phenol derivatives found in certain intravenous medications produce green urine on oxidation. A purple staining may occur in catheter bags and is caused by the presence of indican in the urine or a bacterial infection, frequently caused by *Klebsiella* or *Providencia* species.

■ ■ ● Clarity

Clarity is a general term that refers to the transparency/turbidity of a urine specimen. In routine urinalysis, clarity is determined in the same manner that ancient physicians used: by visually examining the mixed specimen while holding it in front of a light source. The specimen should, of course, be in a clear container. Color and clarity are routinely determined at the same time. Common terminology used to report clarity includes clear, hazy, cloudy, turbid, and milky. As discussed under the section on urine color, terminology should be consistent within a laboratory. A description of urine clarity reporting is presented in Table 4–2.

Normal Clarity

Freshly voided normal urine is usually clear, particularly if it is a midstream clean-catch specimen. Precipitation of amorphous phosphates and carbonates may cause a white cloudiness.

Nonpathologic Turbidity

The presence of squamous epithelial cells and mucus, particularly in specimens from women, can result in a hazy but normal urine.

PROCEDURE



Color and Clarity Procedure

- Use a well-mixed specimen.
- View through a clear container.
- View against a white background.
- Maintain adequate room lighting.
- Evaluate a consistent volume of specimen.
- Determine color and clarity.

Table 4–2	Urine Clarity
Clarity	Term
Clear	No visible particulates, transparent.
Hazy	Few particulates, print easily seen through urine.
Cloudy	Many particulates, print blurred through urine.
Turbid	Print cannot be seen through urine.
Milky	May precipitate or be clotted.

Specimens that are allowed to stand or are refrigerated also may develop turbidity that is nonpathologic. As discussed in Chapter 3, improper preservation of a specimen results in bacterial growth; this increases specimen turbidity but is not representative of the actual specimen.

Refrigerated specimens frequently develop a thick turbidity caused by the precipitation of amorphous phosphates, carbonates, and urates. Amorphous phosphates and carbonates produce a white precipitate in urine with an alkaline pH, whereas amorphous urates produce a precipitate in acidic urine that resembles pink brick dust due to the presence of uroerythyrin.

Additional nonpathologic causes of urine turbidity include semen, fecal contamination, radiographic contrast media, talcum powder, and vaginal creams (Table 4–3).

Pathologic Turbidity

The most commonly encountered pathologic causes of turbidity in a fresh specimen are RBCs, white blood cells (WBCs), and bacteria caused by infection or a systemic organ disorder. Other less frequently encountered causes of pathologic turbidity include abnormal amounts of nonsquamous epithelial cells, yeast, abnormal crystals, lymph fluid, and lipids (Table 4–4).

The clarity of a urine specimen certainly provides a key to the microscopic examination results, because the amount of turbidity should correspond with the amount of material observed under the microscope. Questionable causes

Table 4-3 Nonpathologic Causes of Urine Turbidity

Squamous epithelial cells

Mucus

Amorphous phosphates, carbonates, urates

Semen, spermatozoa

Fecal contamination

Radiographic contrast media

Talcum powder

Vaginal creams

Table 4-4 Pathologic Causes of Urine Turbidity
RBCs
WBCs
Bacteria
Yeast
Nonsquamous epithelial cells
Abnormal crystals
Lymph fluid
Lipids

of urine turbidity can be confirmed by chemical tests shown in Table 4–5.

Clear urine is not always normal. However, with the increased sensitivity of the routine chemical tests, most abnormalities in clear urine will be detected prior to the microscopic analysis. Current criteria used to determine the necessity of performing a microscopic examination on all urine specimens include both clarity and chemical tests for RBCs, WBCs, bacteria, and protein.

■■● Specific Gravity

Lipids

Lymphatic fluid, chyle

The ability of the kidneys to selectively reabsorb essential chemicals and water from the glomerular filtrate is one of the body's most important functions. The intricate process of

Laboratory Correlations Table 4-5 in Urine Turbidity¹¹ Acidic Urine Amorphous urates Radiographic contrast media Alkaline Urine Amorphous phosphates, carbonates Soluble With Heat Amorphous urates, uric acid crystals Soluble in Dilute Acetic Acid RBCs Amorphous phosphates, carbonates Insoluble in Dilute Acetic Acid **WBCs** Bacteria, yeast Spermatozoa Soluble in Ether

46

CHAPTER 4 • Physical Examination of Urine

reabsorption is often the first renal function to become impaired; therefore, an assessment of the kidney's ability to reabsorb is a necessary component of the routine urinalysis. This evaluation can be performed by measuring the specific gravity of the specimen. Specific gravity also detects possible dehydration or abnormalities in antidiuretic hormone and can be used to determine whether specimen concentration is adequate to ensure the accuracy of chemical tests.

Specific gravity is defined as the density of a solution compared with the density of a similar volume of distilled water at a similar temperature. Because urine is actually water that contains dissolved chemicals, the specific gravity of urine is a measure of the density of the dissolved chemicals in the specimen. As a measure of specimen density, specific gravity is influenced not only by the number of particles present but also by their size. Large urea molecules contribute more to the reading than do the small sodium and chloride molecules. Therefore, because urea is of less value than sodium and chloride in the evaluation of renal concentrating ability, it also may be necessary to test the specimen's osmolarity. This procedure is discussed in Chapter 2. For purposes of routine urinalysis, however, the specific gravity provides valuable preliminary information and can be easily performed by direct methods using a urinometer (hydrometer) or harmonic oscillation densitometry (HOD), and by indirect methods using a refractometer or the chemical reagent strip. This chapter will discuss the physical methods for determining specific gravity. The chemical reagent strip method is covered in Chapter 5.

Urinometer

The urinometer consists of a weighted float attached to a scale that has been calibrated in terms of urine specific gravity. The weighted float displaces a volume of liquid equal to its weight and has been designed to sink to a level of 1.000 in distilled water. The additional mass provided by the dissolved substances in urine causes the float to displace a volume of urine smaller than that of distilled water. The level to which the urinometer sinks, as shown in Figure 4-2, represents the specimen's mass or specific gravity.

Urinometry is less accurate than the other methods currently available and is not recommended by the Clinical and Laboratory Standards Institute (CLSI) formerly the National Committee for Clinical Laboratory Standards (NCCLS).⁸ A major disadvantage of using a urinometer to measure specific gravity is that it requires a large volume (10 to 15 mL) of specimen. The container in which the urinometer is floated must be wide enough to allow it to float without touching the sides and deep enough that it does not rest on the bottom. When using the urinometer, an adequate amount of urine is poured into a proper-size container and the urinometer is added with a spinning motion. The scale reading is then taken at the bottom of the urine meniscus.

The urinometer reading may also need to be corrected for temperature, inasmuch as urinometers are calibrated to read 1.000 in distilled water at a particular temperature. The calibration temperature is printed on the instrument and is usu-

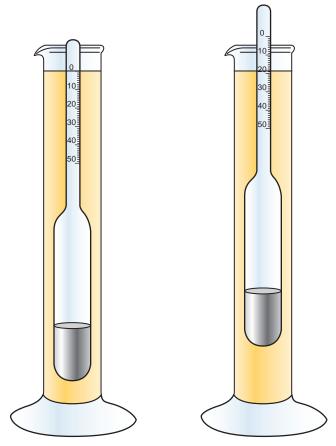


Figure 4–2 Urinometers representing various specific gravity readings.

ally about 20°C. If the specimen is cold, 0.001 must be subtracted from the reading for every 3°C that the specimen temperature is below the urinometer calibration temperature. Conversely, 0.001 must be added to the reading for every 3°C that the specimen measures above the calibration temperature.

A correction also must be calculated when using either the urinometer or the refractometer if large amounts of glucose or protein are present. Both glucose and protein are high-molecular-weight substances that have no relationship to renal concentrating ability but will increase specimen density. Therefore, their contribution to the specific gravity is subtracted to give a more accurate report of the kidney's concentrating ability. A gram of protein per deciliter of urine raises the urine specific gravity by 0.003, and 1 g glucose/dL adds 0.004 to the reading. Consequently, for each gram of protein present, 0.003 must be subtracted from the specific gravity reading, and 0.004 must be subtracted for each gram of glucose present.

Example

A specimen containing 1 g/dL of protein and 1 g/dL of glucose has a specific gravity reading of 1.030. Calculate the corrected reading.

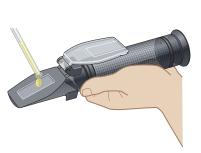
1.030 - 0.003 (protein) = 1.027 - 0.004 (glucose) = 1.023 corrected specific gravity

Refractometer

Refractometry, like urinometry, determines the concentration of dissolved particles in a specimen. It does this by measuring refractive index. Refractive index is a comparison of the velocity of light in air with the velocity of light in a solution. The concentration of dissolved particles present in the solution determines the velocity and angle at which light passes through a solution. Clinical refractometers make use of these principles of light by using a prism to direct a specific (monochromatic) wavelength of daylight against a manufacturer-calibrated specific gravity scale. The concentration of the specimen determines the angle at which the light beam enters the prism. Therefore, the specific gravity scale is calibrated in terms of the angles at which light passes through the specimen.

The refractometer provides the distinct advantage of determining specific gravity using a small volume of specimen (one or two drops). Temperature corrections are not necessary because the light beam passes through a temperature-compensating liquid prior to being directed at the specific gravity scale. Temperature is compensated between 15°C and 38°C. Corrections for glucose and protein are still calculated, although refractometer readings are less affected by particle density than are urinometer readings.

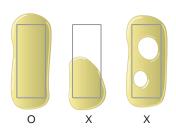
When using the refractometer, a drop of urine is placed on the prism, the instrument is focused at a good light source, and the reading is taken directly from the specific gravity scale. The prism and its cover should be cleaned after each specimen is tested. Figure 4-3 illustrates the use of the refractometer.



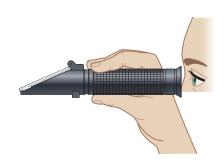
 Put one or two drops of sample on the prism.



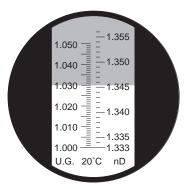
2. Close the daylight plate gently.



3. The sample must spread all over the prism surface.



4. Look at the scale through the eyepiece.



5. Read the scale where the boundary line intercepts it.



6. Wipe the sample from the prism clean with a tissue paper and water.

Figure 4–3 Steps in the use of the urine-specific gravity refractometer. (Courtesy of NSG Precision Cells, Inc., 195G Central Ave., Farmingdale, N.Y., 11735.)

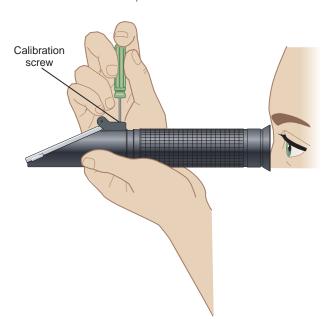


Figure 4–4 Calibration of the urine-specific gravity refractometer. (Courtesy of NSG Precision Cells, Inc., 195G Central Ave., Farmingdale, N.Y., 11735.)

Calibration of the refractometer is performed using distilled water that should read 1.000. If necessary, the instrument contains a zero set screw to adjust the distilled water reading (Fig. 4-4). The calibration is further checked using 5% NaCl, which as shown in the refractometer conversion tables should read 1.022 \pm 0.001, or 9% sucrose that should read 1.034 \pm 0.001. Urine control samples representing low, medium, and high concentrations should also be run at the beginning of each shift. Calibration and control results are always recorded in the appropriate quality control records.

Harmonic Oscillation Densitometry

Harmonic oscillation densitometry is based on the principle that the frequency of a sound wave entering a solution changes in proportion to the density of the solution. Shifts in harmonic oscillation are measured, and relative density is calculated. A portion of the urine sample enters a U-shaped glass tube with an electromagnetic coil at one end and a motion detector at the other end. An electric current is applied to the coil, which causes the sound wave to pass (oscillate) through the urine sample. Its frequency is altered by the density of the specimen. A microprocessor at the other end of the tube measures the change in sound wave frequency, compensates for temperature variations, and converts the reading to specific gravity that closely correlates with gravimetric measurement (Fig. 4-5).8 All dissolved solutes are measured by this method, and there is no clarification of cloudy specimens required. Results are linear up to a specific gravity of 1.080.

Clinical Correlations

The specific gravity of the plasma filtrate entering the glomerulus is 1.010. The term *isosthenuric* is used to describe

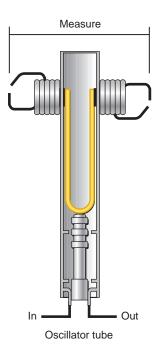


Figure 4–5 Mass gravity meter used to perform specific gravity measurement by harmonic oscillation densitometry. (Courtesy of International Remote Imaging Systems, Chatsworth, Calif.)

urine with a specific gravity of 1.010. Specimens below 1.010 are *hyposthenuric*, and those above 1.010 are *hypersthenuric*. One would expect urine that has been concentrated by the kidneys to be hypersthenuric; however, this is not always true. Normal random specimens may range from 1.003 to 1.035, depending on the patient's amount of hydration. Specimens measuring lower than 1.003 probably are not urine. Most random specimens fall between 1.015 and 1.025, and any random specimen with a specific gravity of 1.023 or higher is generally considered normal. If a patient exhibits consistently low results, specimens may be collected under controlled conditions as discussed in Chapter 2.

Abnormally high results—over 1.035—are seen in patients who have recently undergone an intravenous pyelogram. This is caused by the excretion of the injected radiographic contrast media. Patients who are receiving dextran

Urine Specific Gravity Measurements		
Method	Principle	
Urinometry	Density	
Refractometry	Refractive index	
Harmonic oscillation densitometry	Density	
Reagent strip	pK _a changes of a polyelectrolyte	

or other high-molecular-weight intravenous fluids (plasma expanders) also produce urine with an abnormally high specific gravity. Once the foreign substance has been cleared from the body, the specific gravity returns to normal. In these circumstances, urine concentration can be measured using the reagent strip chemical test or osmometry because they are not affected by these high-molecular-weight substances. When the presence of glucose or protein is the cause of high results, this is detected in the routine chemical examination. As discussed previously, this can be corrected for mathematically.

Specimens with specific gravity readings greater than the refractometer or urinometer scale can be diluted and retested. If this is necessary, only the decimal portion of the observed specific gravity is multiplied by the dilution factor. For example, a specimen diluted 1:2 with a reading of 1.025 would have an actual specific gravity of a 1.050.

Odor

Although it is seldom of clinical significance and is not a part of the routine urinalysis, urine odor is a noticeable physical property. Freshly voided urine has a faint aromatic odor. As the specimen stands, the odor of ammonia becomes more prominent. The breakdown of urea is responsible for the characteristic ammonia odor. Causes of unusual odors include bacterial infections, which cause a strong, unpleasant odor, and diabetic ketones, which produce a sweet or fruity odor. A serious metabolic defect results in urine with a strong odor of maple syrup and is appropriately called maple syrup urine disease. This and other metabolic disorders with characteristic urine odors are discussed in Chapter 9. Ingestion of certain foods, including onions, garlic, and asparagus, can cause an unusual or pungent urine odor. Studies have shown that although everyone who eats asparagus produces an odor, only certain genetically predisposed people can smell the odor. 10 Common causes of urine odors are summarized in Table 4-6.

Table 4-6 Common Causes of Urine Odor ¹¹	
Odor	Cause
Aromatic	Normal
Foul, ammonia-like	Bacterial decomposition, urinary tract infection
Fruity, sweet	Ketones (diabetes mellitus, starvation, vomiting)
Maple syrup	Maple syrup urine disease
Mousy	Phenylketonuria
Rancid	Tyrosinemia
Sweaty feet	Isovaleric acidemia
Cabbage	Methionine malabsorption
Bleach	Contamination

References

- 1. Drabkin, DL: The normal pigment of urine: The relationship of urinary pigment output to diet and metabolism. J Biol Chem 75:443-479, 1927.
- Ostow, M, and Philo, S: The chief urinary pigment: The relationship between the rate of excretion of the yellow pigment and the metabolic rate. Am J Med Sci 207:507-512, 1944.
- 3. Berman, L: When urine is red. JAMA 237:2753-2754, 1977.
- 4. Reimann, HA: Re: Red urine. JAMA 241(22):2380, 1979.
- Evans, B: The greening of urine: Still another "Cloret sign." N Engl J Med 300(4):202, 1979.
- 6. Bowling, P, Belliveau, RR, and Butler, TJ: Intravenous medications and green urine. JAMA 246(3):216, 1981.
- 7. Dealler, SF, et al: Purple urine bags. J Urol 142(3):769-770,
- Clinical and Laboratory Standards Institute (Formerly NCCLS).
 Urinalysis and Collection, Transportation, and Preservation of Urine Specimens; Approved Guideline Second Edition.
 NCCLS document GP16-A2, Wayne, Pa., 2001.
- 9. Smith, C, Arbogast, C, and Phillips, R: Effect of x-ray contrast media on results for relative density of urine. Clin Chem 19(4):730-731, 1983.
- 10. Mitchell, SC, et al: Odorous urine following asparagus ingestion in man. Experimenta 43(4):382-383, 1987.
- Henry, JB, Lauzon, RB, and Schumann, GB: Basic examination of urine. In Henry, JB (ed): Clinical Diagnosis and Management by Laboratory Methods. WB Saunders, Philadelphia, 1996.

QUESTIONS

- **1.** The concentration of a normal urine specimen can be estimated by which of the following?
 - A. Color
 - B. Clarity
 - C. Foam
 - D. Odor
- 2. The normal yellow color of urine is produced by:
 - A. Bilirubin
 - B. Hemoglobin
 - C. Urobilinogen
 - D. Urochrome
- **3.** A yellow-brown specimen that produces a yellow foam when shaken can be suspected of containing:
 - A. Bilirubin
 - B. Carrots
 - C. Hemoglobin
 - D. Rhubarb
- **4.** A urine that turns black after standing may contain:
 - A. Homogentisic acid
 - B. Melanin
 - C. Methemoglobin
 - D. All of the above

©2008 F. A. Davis

Continued

- **5.** Specimens that contain intact RBCs can be visually distinguished from those that contain hemoglobin because:
 - A. Hemoglobin produces a much brighter red color
 - B. Hemoglobin produces a cloudy, pink specimen
 - C. RBCs produce a cloudy specimen
 - D. RBCs are quickly converted to hemoglobin
- 6. After eating beets purchased at the local farmers' market, Mrs. Williams notices that her urine is red, but Mr. William's urine remains yellow. The Williamses should:
 - A. Be concerned because red urine always indicates the presence of blood
 - B. Not be concerned because all women produce red urine after eating beets
 - C. Be concerned because both of them should have red urine if beets are the cause
 - D. Not be concerned because only Mrs. Williams is genetically susceptible to producing red urine from beets
- **7.** Specimens from patients receiving treatment for urinary tract infections frequently appear:
 - A. Clear and red
 - B. Viscous and orange
 - C. Dilute and pale yellow
 - D. Cloudy and red
- **8.** Freshly voided normal urine is usually clear; however, if it is alkaline, a white turbidity may be present due to:
 - A. Amorphous phosphates and carbonates
 - B. Uroerythrin
 - C. WBCs
 - D. Yeast
- **9.** Microscopic examination of a clear urine that produces a pink precipitate after refrigeration will show:
 - A. Amorphous urates
 - B. Porphyrins
 - C. Red blood cells
 - D. Triple phosphate crystals
- **10**. Under what conditions will a port-wine urine color be observed in a urine specimen?
 - A. The patient has eaten Clorets.
 - B. Melanin is present.
 - C. Urine contains porphyrins.
 - D. The patient has a Pseudomonas infection.
- **11.** Which of the following specific gravities would be most likely to correlate with a dark yellow urine?
 - A. 1.005
 - B. 1.010
 - C. 1.020
 - D. 1.030

- **12**. *True or False*: Urine specific gravity is equally influenced by the presence of glucose and sodium.
- **13**. In what circumstance might a sediment be slightly warmed prior to microscopic examination?
 - A. To hemolyze RBCs
 - B. To dissolve amorphous urates
 - C. To increase the specific gravity
 - D. To correct for temperature in determining the specific gravity
- **14.** A urine specific gravity measured by refractometer is 1.029, and the temperataure of the urine is 14°C. The specific gravity should be reported as:
 - A. 1.023
 - B. 1.027
 - C. 1.029
 - D. 1.032
- 15. Refractive index compares:
 - A. Light velocity in solutions with light velocity in solids
 - B. Light velocity in air with light velocity in solutions
 - C. Light scattering by air with light scattering by solutions
 - D. Light scattering by particles in solution
- 16. Refractometers are calibrated using:
 - A. Distilled water and protein
 - B. Distilled water and blood
 - C. Distilled water and sodium chloride
 - D. Distilled water and urea
- 17. A correlation exists between a specific gravity of 1.050 and a:
 - A. 2+ glucose
 - B. 2+ protein
 - C. First morning specimen
 - D. Radiographic dye infusion
- **18.** An alkaline urine turns black upon standing, develops a cloudy white precipitate, and has a specific gravity of 1.012. The major concern about this specimen would be:
 - A. Color
 - B. Turbidity
 - C. Specific gravity
 - D. All of the above
- **19**. The reading of distilled water by the refractometer is 1.003. You should:
 - A. Subtract 1.003 from each specimen reading
 - B. Add 1.003 to each specimen reading
 - C. Use a new refractometer
 - D. Adjust the set screw
- **20.** A urine specimen with a specific gravity of 1.008 has been diluted 1:5. The actual specific gravity is:
 - A. 1.008
 - B. 1.040
 - C. 1.055
 - D. 5.040

- **21.** The method for determining a urine specific gravity that is based on the principle that the frequency of a sound wave entering a solution changes in proportion to the density of the solution is:
 - A. Colorimetric
 - B. Harmonic oscillation densitometry
 - C. Refractometry
 - D. Urinometry
- **22.** A specimen with a specific gravity of 1.005 would be considered:
 - A. Isosthenuric
 - B. Hyposthenuric
 - C. Hypersthenuric
 - D. Not urine
- **23.** *True or False:* Specific gravity is of more diagnostic value than osmolarity in evaluating renal concentration ability.
- **24.** A strong odor of ammonia in a urine specimen could indicate:
 - A. Ketones
 - B. Normal
 - C. Phenylketonuria
 - D. Urinary tract infection
- **25.** The microscopic of a cloudy amber urine is reported as rare WBCs and epithelial cells. What does this suggest?
 - A. Urinary tract infection
 - B. Dilute random specimen
 - C. Precipitated amorphous urates
 - D. Possible mix-up of specimen and sediment
- **26**. A specimen with a strong ammonia odor and a heavy white precipitate when it arrives in the laboratory may require:
 - A. Collection of a fresh specimen
 - B. Centrifugation
 - C. Dilution for specific gravity
 - D. Testing under a hood

Case Studies and Clinical Situations

- 1. A concerned male athlete brings a clear, red urine specimen to the physician's office.
 - a. Would you expect to see RBCs in the microscopic examination? Why or why not?
 - b. Name two pathologic causes of a clear, red urine.

 Under what conditions do these substances appear in the urine?
 - c. The patient reported that the urine appeared cloudy when he collected it the previous evening, but it was clear in the morning. Is this possible? Explain your answer.
 - d. If the urine is chemically negative for blood, what questions should the physician ask the patient?

- **2.** Upon arriving at work, a technologist notices that a urine specimen left beside the sink by personnel on the nightshift has a black color. The initial report describes the specimen as yellow.
 - a. Should the technologist be concerned about this specimen? Explain your answer.
 - b. If the specimen had an initial pH of 6.0 and now has a pH of 8.0, what is the most probable cause of the black color?
 - c. If the specimen has a pH of 6.0 and was sitting uncapped, what is the most probable cause of the black color?
 - d. If the original specimen was reported to be red and to contain RBCs, what is a possible cause of the black color?
- **3.** While performing a routine urinalysis on a specimen collected from a patient in the urology clinic, the technician finds a specific gravity reading that exceeds the 1.035 scale on the refractometer.
 - a. If the urinalysis report has a 1+ protein and a negative glucose, what is the most probable cause of this finding?
 - b. The technician makes a 1:4 dilution of the specimen, repeats the specific gravity, and gets a reading of 1.015. What is the actual specific gravity?
 - c. Using 1 mL of urine, how would the technician make the above dilution?
 - d. How could a specific gravity be obtained from this specimen without diluting it?
- **4.** Mrs. Smith frequently shops at the farmer's market near her home. She notices her urine has a red color and brings a sample to her physician. The specimen tests negative for blood.
 - a. What is a probable cause of Mrs. Smith's red urine?
 - b. Mrs. Smith collects a specimen at the physician's office. The color is yellow and the pH is 5.5. Is this consistent with the previous answer? Why or why not?
- **5.** A urinalysis supervisor requests a new specimen in each of the following situations. Support or disagree with the decisions.
 - a. A green-yellow specimen with negative test results for glucose and bilirubin.
 - b. A dark yellow specimen that produces a large amount of white foam.
 - c. A cloudy urine with a strong odor of ammonia.
 - d. A hazy specimen with a specific gravity greater than 1.035 by refractometer.











Chemical Examination of Urine

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

- 1 Describe the proper technique for performing reagent strip testing.
- **2** List four causes of premature deterioration of reagent strips, and tell how to avoid them.
- **3** List five quality-control procedures routinely performed with reagent strip testing.
- **4** List two reasons for measuring urinary pH, and discuss their clinical applications.
- 5 Discuss the principle of pH testing by reagent strip.
- 6 Differentiate between prerenal, renal, and postrenal proteinuria, and give clinical examples of each.
- 7 Explain the "protein error of indicators," and list any sources of interference that may occur with this method of protein testing.
- **8** Discuss the sulfosalicylic acid (SSA) test for urine protein, including interpretation and sources of interference.
- 9 Describe the unique solubility characteristics of Bence Jones protein, and tell how they can be used to perform a screening test for the presence of this protein.
- 10 Discuss microalbuminuria including significance, reagent strip tests, and their principles.
- 11 Explain why glucose that is normally reabsorbed in the proximal convoluted tubule may appear in the urine, and state the renal threshold levels for glucose.
- 12 Describe the principle of the glucose oxidase method of reagent strip testing for glucose, and name possible causes of interference with this method.

- Describe the copper reduction method for detection of urinary reducing substances, and list possible causes of interference.
- 14 Interpret matching and nonmatching results between the glucose oxidase and the copper reduction tests for glucose.
- 15 Name the three "ketone bodies" appearing in urine and three causes of ketonuria.
- 16 Discuss the principle of the sodium nitroprusside reaction, including sensitivity and possible causes of interference.
- 17 Differentiate between hematuria, hemoglobinuria, and myoglobinuria with regard to the appearance of urine and serum and clinical significance.
- 18 Describe the chemical principle of the reagent strip method for blood testing, and list possible causes of interference.
- 19 Discuss methods used to differentiate between hemoglobinuria and myoglobinuria.
- **20** Outline the steps in the degradation of hemoglobin to bilirubin, urobilinogens, and finally urobilin.
- 21 Describe the relationship of urinary bilirubin and urobilinogen to the diagnosis of bile duct obstruction, liver disease, and hemolytic disorders
- Discuss the principle of the reagent strip test for urinary bilirubin, including possible sources of error.
- 23 Discuss the advantages and disadvantages of performing an Ictotest for detection of urine bilirubin.



LEARNING OBJECTIVES continued

- 24 State two reasons for increased urine urobilinogen and one reason for a decreased urine urobilinogen.
- 25 Describe the Watson-Schwartz test used to differentiate among urobilinogen, porphobilinogen, Ehrlich reactive compounds, and the Hoesch screening test for porphobilinogen.
- **26** Discuss the principle of the nitrite-reagent-strip test for bacteriuria.
- 27 List five possible causes of a false-negative result in the reagent-strip test for nitrite.

- **28** State the principle of the reagent strip test for leukocytes.
- **29** Discuss the advantages and sources of error of the reagent strip test for leukocytes.
- **30** Explain the principle of the chemical test for specific gravity.
- 31 Compare reagent strip testing for urine specific gravity with urinometer and refractometer testing.
- **32** Correlate physical and chemical urinalysis results.

KEY TERMS

bacteriuria
bilirubin
glycosuria
hematuria
hemoglobinuria
ketonuria

leukocyturia microalbuminuria myoglobinuria orthostatic proteinuria postrenal proteinuria prerenal proteinuria protein error of indicators proteinuria renal proteinuria stercobilinogen urobilinogen

■ ■ ■ Reagent Strips

Routine chemical examination of urine has changed dramatically since the early days of urine testing, owing to the development of the reagent strip method for chemical analysis. Reagent strips currently provide a simple, rapid means for performing medically significant chemical analysis of urine, including pH, protein, glucose, ketones, blood, bilirubin, urobilinogen, nitrite, leukocytes, and specific gravity. The two major types of reagent strips are manufactured under the tradenames Multistix (Siemens Medical Solutions Diagnostics, Tarrytown, N.Y.) and Chemstrip (Roche Diagnostics, Indianapolis, Ind.). These products are available with singleor multiple-testing areas, and the brand and number of tests used are a matter of laboratory preference. Certain variations relating to chemical reactions, sensitivity, specificity, and interfering substances occur among the products and are discussed in the following sections. Reagent strip brands are also specified by instrumentation manufacturers.

Reagent strips consist of chemical-impregnated absorbent pads attached to a plastic strip. A color-producing chemical reaction takes place when the absorbent pad comes in contact with urine. The reactions are interpreted by comparing the color produced on the pad with a chart supplied by the manufacturer. Several colors or intensities of a color for each substance being tested appear on the chart. By careful comparison of the colors on the chart and the strip, a semiquantitative value of trace, 1+, 2+, 3+, or 4+ can be

reported. An estimate of the milligrams per deciliter present is available for appropriate testing areas. Automated reagent strip readers also provide Système International units.

Reagent Strip Technique

Testing methodology includes dipping the reagent strip completely, but briefly, into a well-mixed specimen, removing excess urine from the strip by running the edge of the strip on the container when withdrawing it from the specimen, waiting the specified length of time for reactions to take place, and comparing the colored reactions against the manufacturer's chart using a good light source.

Improper technique can result in errors. Formed elements such as red and white blood cells sink to the bottom of the specimen and will be undetected in an unmixed specimen. Allowing the strip to remain in the urine for an extended period may cause leaching of reagents from the pads. Likewise, excess urine remaining on the strip after its removal from the specimen can produce a runover between chemicals on adjacent pads, producing distortion of the colors. To ensure against runover, blotting the edge of the strip on absorbent paper and holding the strip horizontally while comparing it with the color chart is recommended. The amount of time needed for reactions to take place varies between tests and manufacturers, and ranges from an immediate reaction for pH to 120 seconds for leukocytes. For the best semiquantitative results, the manufacturer's stated time should be fol-

PROCEDURE

Reagent Strip Technique

Dip the reagent strip briefly into a well-mixed uncentrifuged urine specimen at room temperature.

Remove excess urine by touching the edge of the strip to the container as the strip is withdrawn.

Blot the edge of the strip on a disposable absorbent pad. Wait the specified amount of time for the reaction to occur.

Compare the color reaction of the strip pads to the manufacturer's color chart in good lighting.

lowed; however, when precise timing cannot be achieved, manufacturers recommend that reactions be read between 60 and 120 seconds, with the leukocyte reaction read at 120 seconds. A good light source is, of course, essential for accurate interpretation of color reactions. The strip must be held close to the color chart without actually being placed on the chart. Automated reagent strip instruments standardize the color interpretation and timing of the reaction and are not subject to room lighting deficiencies or inconsistency among laboratory personnel (Appendix A). Reagent strips and color charts from different manufacturers are not interchangeable. Specimens that have been refrigerated must be allowed to return to room temperature prior to reagent strip testing, as the enzymatic reactions on the strips are temperature dependent.

Handling and Storage of Reagent Strips

In addition to using correct testing technique, reagent strips must be protected from deterioration caused by moisture, volatile chemicals, heat, and light. Reagent strips are packaged in opaque containers with a desiccant to protect them from light and moisture. Strips are removed just prior to testing, and the bottle is tightly resealed immediately. Bottles should not be opened in the presence of volatile fumes. Manufacturers recommend that reagent strips be stored at room temperature below 30°C. All bottles are stamped with an expiration date that represents the functional life expectancy of the chemical pads. Reagent strips must not be used past the expiration date. Care must be taken not to touch the chemical pads when removing the strips.

Quality Control of Reagent Strips

Reagent strips must be checked with both positive and negative controls a minimum of once every 24 hours. Many laboratories perform this check at the beginning of each shift. Testing is also performed when a new bottle of reagent strips is opened, questionable results are obtained, or there is concern about the integrity of the strips. All quality control results must be recorded following laboratory protocol. Several com-

Summary of Reagent Strip Testing

Care of Reagent Strips

- I. Store with desiccant in an opaque, tightly closed container.
- 2. Store below 30°C; do not freeze.
- 3. Do not expose to volatile fumes.
- 4. Do not use past the expiration date.
- 5. Do not use if chemical pads become discolored.
- 6. Remove strips immediately prior to use.

Technique

- I. Mix specimen well.
- 2. Let refrigerated specimens warm to room temperature before testing.
- 3. Dip the strip completely, but briefly, into specimen.
- 4. Remove excess urine by withdrawing the strip against the rim of the container and by blotting the edge of the strip.
- 5. Compare reaction colors with the manufacturer's chart under a good light source at the specified time.
- 6. Perform backup tests when indicated.
- 7. Be alert for the presence of interfering substances.
- 8. Understand the principles and significance of the test, read package inserts.
- 9. Relate chemical findings to each other and to the physical and microscopic urinalysis results.

Quality Control

- I.Test open bottles of reagent strips with known positive and negative controls every 24 hr.
- 2. Resolve control results that are out of range by further testing.
- 3. Test reagents used in backup tests with positive and negative controls.
- 4. Perform positive and negative controls on new reagents and newly opened bottles of reagent strips.
- 5. Record all control results and reagent lot numbers.

panies manufacture both positive and negative controls. Distilled water is not recommended as a negative control because reagent strip chemical reactions are designed to perform at ionic concentrations similar to urine. 1 All readings of the negative control must be negative, and positive control readings should agree with the published value by \pm one color block. Results that do not agree with the published values must be resolved through the testing of additional strips and controls (see Chapter 7).

Demonstration of chemically acceptable reagent strips does not entirely rule out the possibility of inaccurate results. Interfering substances in the urine, technical carelessness, and color blindness also produce errors. Reagent strip manufacturers have published information concerning the limitations of their chemical reactions, and laboratory personnel should

56 CHAPTER 5 • Chemical Examination of Urine

be aware of these conditions. As mentioned in Chapter 4, a primary example of reagent strip interference is the masking of color reactions by the orange pigment present in the urine of persons taking phenazopyridine compounds. If laboratory personnel do not recognize the presence of this pigment or other pigments, they will report many erroneous results.

Nonreagent strip testing procedures using tablets and liquid chemicals are available when questionable results are obtained or highly pigmented specimens are encountered. In the past, many of these procedures were used routinely to confirm positive results. Increased specificity and sensitivity of reagent strips and the use of automated strip readers have reduced the need for routine use of these procedures.² The chemical reliability of these procedures also must be checked using positive and negative controls. Specific backup tests are discussed in this chapter under the sections devoted to the chemical parameters for which they are used.

■■● pH

Along with the lungs, the kidneys are the major regulators of the acid-base content in the body. They do this through the secretion of hydrogen in the form of ammonium ions, hydrogen phosphate, and weak organic acids, and by the reabsorption of bicarbonate from the filtrate in the convoluted tubules (see Chapter 2). A healthy individual usually produces a first morning specimen with a slightly acidic pH of 5.0 to 6.0; a more alkaline pH is found following meals (alkaline tide). The pH of normal random samples can range from 4.5 to 8.0. Consequently, no normal values are assigned to urinary pH, and it must be considered in conjunction with other patient information, such as the acid-base content of the blood, the patient's renal function, the presence of a urinary tract infection, the patient's dietary intake, and the age of the specimen (Table 5–1).

Clinical Significance

The importance of urinary pH is primarily as an aid in determining the existence of systemic acid-base disorders of metabolic or respiratory origin and in the management of urinary conditions that require the urine to be maintained at a specific pH. In respiratory or metabolic acidosis not related to renal function disorders, the urine is acidic; conversely, if respiratory or metabolic alkalosis is present, the urine is alkaline. Therefore, a urinary pH that does not conform to this pattern may be used to rule out the suspected condition, or, as discussed in Chapter 2, it may indicate a disorder resulting from the kidneys' inability to secrete or to reabsorb acid or base.

The precipitation of inorganic chemicals dissolved in the urine forms urinary crystals and renal calculi. This precipitation depends on urinary pH and can be controlled by maintaining the urine at a pH that is incompatible with the precipitation of the particular chemicals causing the calculi formation. For example, calcium oxalate, a frequent constituent of renal calculi, precipitates primarily in acidic and

Table 5-1 Causes of Acid and Alkaline Urine		
Acid Urine	Alkaline Urine	
Emphysema	Hyperventilation	
Diabetes mellitus	Vomiting	
Starvation	Renal tubular acidosis	
Dehydration	Presence of urease- producing bacteria	
Diarrhea	Vegetarian diet	
Presence of acid- producing bacteria	Old specimens	
(Escherichia coli)		
High-protein diet		
Cranberry juice		
Medications (methenamine mandelate [Mandelamine], fosfomycin tromethamine)		

not alkaline urine. Therefore, maintaining urine at an alkaline pH discourages formation of the calculi. Knowledge of urinary pH is important in the identification of crystals observed during microscopic examination of the urine sediment. This will be discussed in detail in Chapter 6.

The maintenance of an acidic urine can be of value in the treatment of urinary tract infections caused by urea-splitting organisms because they do not multiply as readily in an acidic medium. These same organisms are also responsible for the highly alkaline pH found in specimens that have been allowed to sit unpreserved for extended periods. Urinary pH is controlled primarily by dietary regulation, although medications also may be used. Persons on high-protein and highmeat diets tend to produce acidic urine, whereas urine from vegetarians is more alkaline, owing to the formation of bicarbonate following digestion of many fruits and vegetables. An exception to the rule is cranberry juice, which produces an acidic urine and has long been used as a home remedy for minor bladder infections. Medications prescribed for urinary tract infections, such as methenamine mandelate (Mandelamine) and fosfomycin tromethamine, are metabolized to produce an acidic urine.

The pH of freshly excreted urine does not reach 9 in normal or abnormal conditions. A pH of 9 is associated with an improperly preserved specimen and indicates that a fresh specimen should be obtained to ensure the validity of the analysis.

Reagent Strip Reactions

The Multistix and Chemstrip brands of reagent strips measure urine pH in 0.5- or 1-unit increments between pH 5 and 9.

Summary of Clinical Significance of Urine pH

- 1. Respiratory or metabolic acidosis/ketosis
- 2. Respiratory or metabolic alkalosis
- 3. Defects in renal tubular secretion and reabsorption of acids and bases—renal tubular acidosis
- 4. Renal calculi formation
- 5. Treatment of urinary tract infections
- 6. Precipitation/identification of crystals
- 7. Determination of unsatisfactory specimens

To differentiate pH units throughout this wide range, both manufacturers use a double-indicator system of methyl red and bromthymol blue. Methyl red produces a color change from red to yellow in the pH range 4 to 6, and bromthymol blue turns from yellow to blue in the range of 6 to 9. Therefore, in the pH range 5 to 9 measured by the reagent strips, one sees colors progressing from orange at pH 5 through yellow and green to a final deep blue at pH 9.

Methyl red + $H^+ \rightarrow$ Bromthymol blue - H^+ (Red-Orange \rightarrow Yellow) (Green \rightarrow Blue)

No known substances interfere with urinary pH measurements performed by reagent strips. Care must be taken, however, to prevent runover between the pH testing area and the adjacent, highly acidic protein testing area on Multistix, as this may produce a falsely acidic reading in an alkaline urine.

Protein

Of the routine chemical tests performed on urine, the most indicative of renal disease is the protein determination. The presence of proteinuria is often associated with early renal disease, making the urinary protein test an important part of any physical examination. Normal urine contains very little protein: usually, less than 10 mg/dL or 100 mg per 24 hours is excreted. This protein consists primarily of low-molecular-

Summary of pH Reagent Strip Reagents Methyl red, bromthymol blue Sensitivity 5–9 Sources of error/ interference Runover from adjacent pads Old specimens Correlations with other tests Leukocytes Microscopic

weight serum proteins that have been filtered by the glomerulus and proteins produced in the genitourinary tract. Owing to its low molecular weight, albumin is the major serum protein found in normal urine. Even though it is present in high concentrations in the plasma, the normal urinary albumin content is low because the majority of albumin presented to the glomerulus is not filtered, and much of the filtered albumin is reabsorbed by the tubules. Other proteins include small amounts of serum and tubular microglobulins, Tamm-Horsfall protein produced by the tubules, and proteins from prostatic, seminal, and vaginal secretions.

Clinical Significance

Demonstration of proteinuria in a routine analysis does not always signify renal disease; however, its presence does require additional testing to determine whether the protein represents a normal or a pathologic condition. Clinical proteinuria is indicated at ≥30 mg/dL (300 mg/L).³ The causes of proteinuria are varied and can be grouped into three major categories: *pre-renal*, *renal*, and *postrenal*, based on the origin of the protein.

Prerenal Proteinuria

As the name implies, prerenal proteinuria is caused by conditions affecting the plasma prior to its reaching the kidney and, therefore, is not indicative of actual renal disease. This condition is frequently transient, caused by increased levels of low-molecular-weight plasma proteins such as hemoglobin, myoglobin, and the *acute phase reactants* associated with infection and inflammation. The increased filtration of these proteins exceeds the normal reabsorptive capacity of the renal tubules, resulting in an overflow of the proteins into the urine. Because reagent strips detect primarily albumin, prerenal proteinuria is usually not discovered in a routine urinalysis.

Bence Jones Protein

A primary example of proteinuria due to increased serum protein levels is the excretion of Bence Jones protein by persons with multiple myeloma. In multiple myeloma, a proliferative disorder of the immunoglobulin-producing plasma cells, the serum contains markedly elevated levels of monoclonal immunoglobulin light chains (Bence Jones protein). This low-molecular-weight protein is filtered in quantities exceeding the tubular reabsorption capacity and is excreted in the urine.

When Bence Jones protein is suspected, a screening test that uses the unique solubility characteristics of the protein can be performed. Unlike other proteins, which coagulate and remain coagulated when exposed to heat, Bence Jones protein coagulates at temperatures between 40°C and 60°C and dissolves when the temperature reaches 100°C. Therefore, a specimen that appears turbid between 40°C and 60°C and clear at 100°C can be suspected of containing Bence Jones protein. Interference due to other precipitated proteins can be removed by filtering the specimen at 100°C and observing the specimen for turbidity as it cools to between 40°C and 60°C. Not all per-

58

sons with multiple myeloma excrete detectable levels of Bence Jones protein. Suspected cases of multiple myeloma must be diagnosed by performing serum electrophoresis and immunoelectrophoresis.

Renal Proteinuria

Proteinuria associated with true renal disease may be the result of either glomerular or tubular damage.

Glomerular Proteinuria

When the glomerular membrane is damaged, selective filtration is impaired, and increased amounts of serum protein and eventually red and white blood cells pass through the membrane and are excreted in the urine. Conditions that present the glomerular membrane with abnormal substances (e.g., *amyloid material*, toxic substances, and the immune complexes found in lupus erythematosus and streptococcal glomerulonephritis) are major causes of proteinuria due to glomerular damage.

Increased pressure from the blood entering the glomerulus may override the selective filtration of the glomerulus, causing increased albumin to enter the filtrate. This condition may be reversible, such as occurs during strenuous exercise and dehydration or associated with hypertension. Proteinuria that occurs during the latter months of pregnancy may indicate a pre-eclamptic state and should be considered in conjunction with other clinical symptoms, such as hypertension, to determine if this condition exists.

Tubular Proteinuria

Increased albumin is also present in disorders affecting tubular reabsorption because the normally filtered albumin can no longer be reabsorbed. Other low-molecular-weight proteins that are usually reabsorbed are also present. Causes of tubular dysfunction include exposure to toxic substances and heavy metals, severe viral infections, and *Fanconi syndrome*. The amount of protein that appears in the urine following glomerular damage ranges from slightly above normal to 4 g/day, whereas markedly elevated protein levels are seldom seen in tubular disorders.

The discovery of protein, particularly in a random sample, is not always of pathologic significance, because several benign causes of renal proteinuria exist. Benign proteinuria is usually transient and can be produced by conditions such as strenuous exercise, high fever, dehydration, and exposure to cold.

Orthostatic (Postural) Proteinuria

A persistent benign proteinuria occurs frequently in young adults and is termed *orthostatic*, or postural, proteinuria. It occurs following periods spent in a vertical posture and disappears when a horizontal position is assumed. Increased pressure on the renal vein when in the vertical position is believed

to account for this condition. Patients suspected of orthostatic proteinuria are requested to empty their bladder before going to bed, collect a specimen immediately upon arising in the morning, and collect a second specimen after remaining in a vertical position for several hours. Both specimens are tested for protein, and if orthostatic proteinuria is present, a negative reading will be seen on the first morning specimen, and a positive result will be found on the second specimen.

Microalbuminuria

The development of diabetic nephropathy leading to reduced glomerular filtration and eventual renal failure is a common occurrence in persons with both type 1 and type 2 diabetes mellitus. Onset of renal complications can first be predicted by detection of *microalbuminuria*, and the progression of renal disease can be prevented through better stabilization of blood glucose levels and controlling of hypertension. The presence of microalbuminuria is also associated with an increased risk of cardiovascular disease.^{4,5}

Prior to the development of current reagent strip methods that are specific for albumin, detection of microalbuminuria required collection of a 24-hr urine specimen. Specimens were tested using quantitative procedures for albumin. Results were reported in mg of albumin/24 hours or as the albumin excretion (AER) in μ g/min. With these methods, microalbumin is considered significant when 30 to 300 mg of albumin is excreted in 24 hours or the AER is 20-200 μ g/min.

Postrenal Proteinuria

Protein can be added to a urine specimen as it passes through the structures of the lower urinary tract (ureters, bladder, urethra, prostate, and vagina). Bacterial and fungal infections and inflammations produce exudates containing protein from the interstitial fluid. The presence of blood as the result of injury or menstrual contamination contributes protein, as does the presence of prostatic fluid and large amounts of spermatozoa.

Reagent Strip Reactions

Traditional reagent strip testing for protein uses the principle of the *protein error of indicators* to produce a visible colorimetric reaction. Contrary to the general belief that indicators produce specific colors in response to particular pH levels, certain indicators change color in the presence of protein even though the pH of the medium remains constant. This is because protein (primarily albumin) accepts hydrogen ions from the indicator. The test is more sensitive to albumin because albumin contains more amino groups to accept the hydrogen ions than other proteins. Depending on the manufacturer, the protein area of the strip contains either tetrabromphenol blue or 3′, 3″, 5′, 5″-tetrachlorophenol-3, 4, 5, 6-tetrabromosulfonphthalein and an acid buffer to maintain the pH at a constant level. At a pH level of 3, both indicators appear yellow in the absence of protein; however, as the pro-

Summary of Clinical Significance of Urine Protein

Prerenal	Tubular Disorders
Intravascular hemolysis	Fanconi syndrome
Muscle injury	Toxic agents/heavy metals
Acute phase reactants	Severe viral infections
Multiple myeloma	
Renal	Postrenal
Glomerular disorders	Lower urinary tract infections/ inflammation
lmmune complex disorders	Injury/trauma
	Menstrual contamination
Amyloidosis	Prostatic fluid/spermatozoa
Toxic agents Diabetic nephropathy Strenuous exercise Dehydration Hypertension Pre-eclampsia Orthostatic or postural p	Vaginal secretions roteinuria

tein concentration increases, the color progresses through various shades of green and finally to blue. Readings are reported in terms of negative, trace, 1+, 2+, 3+, and 4+; or the semiquantitative values of 30, 100, 300, or 2000 mg/dL corresponding to each color change. Trace values are considered to be less than 30 mg/dL. Interpretation of trace readings can be difficult. Reporting of trace values may be a laboratory option. The specific gravity of the specimen should be considered because a trace protein in a dilute specimen is more significant than in a concentrated specimen.

Indicator + Protein
$$\xrightarrow{\text{pH } 3.0}$$
 Protein + H⁺
(Yellow) Indicator - H⁺
(Blue-green)

Reaction Interference

The major source of error with reagent strips occurs with highly buffered alkaline urine that overrides the acid buffer system, producing a rise in pH and a color change unrelated to protein concentration. Likewise, a technical error of allowing the reagent pad to remain in contact with the urine for a prolonged period may remove the buffer. False-positive read-

ings are obtained when the reaction does not take place under acidic conditions. Highly pigmented urine and contamination of the container with quaternary ammonium compounds, detergents, and antiseptics also cause false-positive readings. A false-positive trace reading may occur in specimens with a high specific gravity. The fact that reagent strips detect primarily albumin can result in a false-negative reading in the presence of proteins other than albumin.

Traditionally, most laboratories chose to confirm all positive protein results using the sulfosalicyclic acid (SSA) precipitation test. This practice is being replaced by more selective criteria to determine the need for additional testing. For example, some laboratories perform SSA testing only on highly alkaline urines, and others acidify the specimen and retest using a reagent strip. Also, a laboratory with an automated strip reader can opt not to record trace readings.

Sulfosalicylic Acid Precipitation Test

The SSA test is a cold precipitation test that reacts equally with all forms of protein (Table 5–2). Various concentrations

Summary of	of Protein Reagent Strip
Reagents	Multistix: Tetrabromphenol blue Chemstrip: 3', 3", 5', 5" tetra- chlorophenol 3, 4, 5, 6-tetrabromosul- fophthalein
Sensitivity	Multistix: 15–30 mg/dL albumin Chemstrip: 6 mg/dL albumin
Sources of error/ interference	False-positive: Highly buffered alkaline urine Pigmented specimens, phenazopyridine Quaternary ammonium compounds (detergents) Antiseptics, chlorhexidine Loss of buffer from prolonged exposure of the reagent strip to the specimen High specific gravity False-negative: Proteins other than albumin Microalbuminuria
Correlations with other tests	Blood Nitrite Leukocytes Microscopic

PROCEDURE

Sulfosalicylic Acid (SSA) Precipitation Test

- Add 3 mL of 3% SSA reagent to 3 mL of centrifuged
- Mix by inversion and observe for cloudiness.
- Grade the degree of turbidity (see Table 5-2).

Microalbumin Testing Summary

Immunologic Tests

Micral-Test

Principle: Enzyme immunoassay

Sensitivity: 0–10 mg/dL

Reagents: Gold-labeled antibody

B-galactosidase

Chlorophenol red galactoside Interference: False negative: Dilute urine

Immunodip

Principle: Immunochromographics

Sensitivity: 1.2–8.0 mg/dL

Reagents: Antibody coated blue latex particles Interference: False negative-dilute urine

Albumin: Creatinine Ratio

Clinitest Microalbumin Strips/Multistix-Pro

Principle: Sensitive albumin tests related to creatinine concentration to correct for patient hydration Reagents:

Albumin: diodo-dihydroxydinitrophenyl tetrabromosulfonphtalein

Creatinine: copper sulfate, tetramethylbenzidine, diisopropylbenzenedihydroperoxide

Sensitivity:

Albumin: 10-150 mg/L

Creatinine: 10-300 mg/dL, 0.9-26.5 mmol/L

Interference:

Visibly bloody or abnormally colored urine Creatinine: Cimetidine-False Positive

and amounts of SSA can be used to precipitate protein, and methods vary greatly among laboratories. All precipitation tests must be performed on centrifuged specimens to remove any extraneous contamination.

Of course, any substance precipitated by acid produces false turbidity in the SSA test. The most frequently encountered substances are radiographic dyes, tolbutamide metabolites, cephalosporins, penicillins, and sulfonamides.⁶ The presence of radiographic material can be suspected when a markedly elevated specific gravity is obtained. In the presence of radiographic dye, the turbidity also increases on standing due to the precipitation of crystals rather than protein. The patient's history provides the necessary information on tolbutamide and antibiotic ingestion. In contrast to the reagent

Table 5–	2 Reportin	g SSA Turbidity
Grade	Turbidity	Protein Range (mg/dL)
Negative	No increase in turbidity	<6
Trace	Noticeable tur- bidity	6–30
1+	Distinct turbidity with no granulation	30–100
2+	Turbidity with granulation with no flocculation	100–200
3+	Turbidity with granulation and flocculation	200–400
4+	Clumps of protein	>400

strip test, a highly alkaline urine produces false-negative readings in precipitation tests, as the higher pH interferes with precipitation. Use of a more concentrated solution of SSA may overcome the effect of a highly buffered, alkaline urine.

Testing for Microalbuminuria

Several semiquantitative reagent strip methods have been developed so that patients at risk for renal disease can be monitored using random or first morning specimens. These methods are based on immunochemical assays for albumin or albumin-specific reagent strips that also measure creatinine to produce an albumin:creatinine ratio.

Immunochemical assays include the Micral-Test (Roche Diagnostics, Indianapolis, Ind.) and the Immunodip (Diagnostic Chemicals Limited, Oxford, Canada). Both reagent strips are read visually, and first morning specimens are recommended.

Micral-Test reagent strips contain a gold-labeled antihuman albumin antibody-enzyme conjugate. Strips are dipped into the urine up to a level marked on the strip and held for 5 seconds. Albumin in the urine binds to the antibody. The bound and unbound conjugates move up the strip by wicking action. Unbound conjugates are removed in a captive zone by combining with albumin embedded in the strip. The urine albumin-bound conjugates continue up the strip and reach an area containing enzyme substrate. The conjugated enzyme reacts with the substrate, producing colors ranging from white to red. The amount of color produced represents the amount of albumin present in the urine. The color is compared with a chart on the reagent strip bottle after 1 minute. Results range from 0 to 10 mg/dL.

The Immunodip reagent strip uses an immunochromographic technique. Strips are individually packaged in specially designed containers. The container is placed in the urine specimen for 3 minutes. A controlled amount of urine enters the container through a vent hole. The urine encounters blue latex particles coated with antihuman albumin antibody. Albumin in the urine binds with the coated particles. The bound and unbound particles continue to migrate up the strip. The migration is controlled by the size of the particles; unbound particles do not migrate as far as the bound particles. First a blue band is formed by the unbound particles. The bound particles continue to migrate and form a second blue band further up the strip. The top band therefore represents the bound particles (urine albumin) and the bottom band represents unbound particles. The color intensity of the bands is compared against the manufacturer's color chart. A darker bottom band represents greater than 1.2 mg/dL, equal band colors represent 1.2 to 1.8 mg/dL, and a darker top band represents 2.0 to 8.0 mg/dL of albumin. A darker bottom band is negative, equal band color is borderline, and a darker top band represents positive results.

Albumin: Creatinine Ratio

The Clinitek Microalbumin reagent strips and the Multistix Pro reagent strips (Siemens Medical Solutions Diagnostics, Tarrytown, N.Y.) provide simultaneous measurement of albumin/protein and creatinine that permits an estimation of the 24-hr microalbumin excretion.³ As discussed in Chapter 2, creatinine is produced and excreted at a consistent rate for each individual. Therefore, by comparing the albumin excretion to the creatinine excretion, the albumin reading can be corrected for overhydration and dehydration in a random sample. In additon to including creatinine on the reagent strip, the albumin test pad is changed to a dye-binding reaction that is more specific for albumin than the protein error of indicators reaction on strips measuring protein.

Reagent Strip Reactions

Albumin

Albumin reagent strips utilize the dye bis(3',3", diodo-4',4"dihydroxy-5',5"-dinitrophenyl)-3,4,5,6-tetra-bromosulphonphtalein (DIDNTB), which has a higher sensitivity and specificity for albumin. Whereas conventional protein reagent pads have a sensitivity ≥30 mg/dL and may include proteins other than albumin, the DIDNTB strips can measure albumin between 8 and 20 mg/dL (80 to 200 mg/L) without inclusion of other proteins. Reaction interference by highly buffered alkaline urine (always a concern with conventional reagent strips) is controlled by using paper treated with bis-(heptapropylene glycol) carbonate. Addition of polymethyl vinyl ether decreases the nonspecific binding of polyamino acids to the albumin pad. Results are reported as 10 to 150 mg/L (1 to 15 mg/dL). Colors range from pale green to aqua blue. Falsely elevated results can be caused by visibly bloody urine, and abnormally colored urines may interfere with the readings.⁷ Creatinine

The principle of the reagent strip for creatinine is based on the pseudoperoxidase activity of copper-creatinine complexes. The reaction follows the same principle as the reaction for blood on the reagent strips. Reagent strips contain copper sulfate (CuSO₄, 3,3′,5,5′-tetramethylbenzidine (TMB) and disopropyl benzene dihydroperoxide (DBDH). Creatinine in the

urine combines with the copper sulfate to form coppercreatinine peroxidase. This reacts with the peroxide DBDH, releasing oxygen ions that oxidize the chromogen TMB and producing a color change from orange through green to blue.³

$$\begin{array}{c} \text{CuSO}_4 + \text{CRE} \rightarrow \text{Cu(CRE) peroxidase} \\ \text{DBDH} + \text{TMB} \xrightarrow{\text{Cu(CRE) peroxidase}} \text{Oxidized TMB} + \text{H}_2\text{O} \\ \text{(peroxidase) (chromogen)} \end{array}$$

Results are reported as 10, 50, 100, 200, 300 mg/dL or 0.9, 4.4, 8.8, 17.7, or 26.5 mmol/L of creatinine.

Reagent strips are unable to detect the absence of creatinine. Falsely elevated results can be caused by visibly bloody urine and the presence of the gastric acid—reducing medication cimetidine (Tagamet). Abnormally colored urines also may interfere with the readings.

No creatinine readings are considered abnormal, as creatinine is normally present in concentrations of 10 to 300 mg/dL (0.9 to 26.5 mmol/L). The purpose of the creatinine measurement is to correlate the albumin concentration to the urine concentration, producing a semiquantitative albumin: creatinine ratio (A:C) ratio.

Albumin/Protein:Creatinine Ratio

Automated and manual methods are available for determining the A:C ratio based on the previously discussed reactions. The Clinitek Microalbumin reagent strips are designed for instrumental use only. Strips are read on Clinitek Urine Chemistry Analyzers. The strips measure only albumin and creatinine and calculate the A:C ratio. Results are displayed and printed out for albumin, creatinine, and the A:C ratio in both conventional and S.I. units. Abnormal results for the A:C ratio are 30 to 300 mg/g or 3.4 to 33.9 mg/mmol.⁷

The Bayer Multistix Pro 11 reagent strips include reagent pads for creatinine, protein-high and protein-low (albumin), along with pads for glucose, ketones, blood, nitrite, leukocyte esterase, pH, bilirubin, and specific gravity. Urobilinogen is not included on these strips. The strips can be read manually or on automated Clinitek instruments. The protein-high reaction uses the protein error of indicators principle and the protein-low reaction is the previously discussed dye-binding method. Results are reported as the protein:creatinine ratio, although the protein-low result is also included in the calculation. A manufacturer-supplied chart is used to determine the ratio based on the results of the protein-high, protein-low, and creatinine readings.⁸

Glucose

Because of its value in the detection and monitoring of diabetes mellitus, the glucose test is the most frequent chemical analysis performed on urine. Owing to the nonspecific symptoms associated with the onset of diabetes, it is estimated that more than half of the cases in the world are undiagnosed. Therefore, blood and urine glucose tests are included in all physical examinations and are often the focus of mass health screening programs. Early diagnosis of diabetes mellitus through blood and urine glucose tests provides a greatly improved prognosis. Using currently available reagent strip

62 CHAPTER 5 • Chemical Examination of Urine

methods for both blood and urine glucose testing, patients can monitor themselves at home and can detect regulatory problems prior to the development of serious complications.

Clinical Significance

Under normal circumstances, almost all the glucose filtered by the glomerulus is reabsorbed in the proximal convoluted tubule; therefore, urine contains only minute amounts of glucose. Tubular reabsorption of glucose is by active transport in response to the body's need to maintain an adequate concentration of glucose. Should the blood level of glucose become elevated (hyperglycemia), as occurs in diabetes mellitus, the tubular transport of glucose ceases, and glucose appears in the urine. The blood level at which tubular reabsorption stops (renal threshold) for glucose is approximately 160 to 180 mg/dL. Blood glucose levels fluctuate, and a normal person may have glycosuria following a meal with a high glucose content. Therefore, the most informative glucose results are obtained from specimens collected under controlled conditions. Fasting prior to the collection of samples for screening tests is recommended. For purposes of diabetes monitoring, specimens are usually tested 2 hours after meals. A first morning specimen does not always represent a fasting specimen because glucose from an evening meal may remain in the bladder overnight, and patients should be advised to empty the bladder and collect the second specimen. 9 Urine for glucose testing is sometimes collected in conjunction with the blood samples drawn during the course of a glucose tolerance test that is used to confirm the diagnosis of diabetes mellitus or hypoglycemia.

Hyperglycemia that occurs during pregnancy and disappears after delivery is called gestational diabetes. The onset of the hyperglycemia and glycosuria is normally around the sixth month of pregnancy. Hormones secreted by the placenta block the action of insulin, resulting in insulin resistance and hyperglycemia. Detection of gestational diabetes is important to the welfare of the baby, because glucose crosses the placenta whereas insulin does not. The baby develops

Summary of Clinical Significance of Urine Glucose

Hyperglycemia-Associated

Diabetes mellitus Pancreatitis

Pancreatic cancer

Acromegaly

Cushing syndrome Hyperthyroidism

Pheochromocytoma

Central nervous system damage

Stress

Gestational diabetes

Renal-Associated

Fanconi syndrome Advanced renal disease Osteomalacia Pregnancy high glucose levels, causing the baby's pancreas to produce more insulin. The excess glucose presented to the baby is stored as fat, resulting in a large baby at risk for obesity and later type 2 diabetes. Women who have gestational diabetes also are prone to developing type 2 diabetes mellitus in later years.

Hyperglycemia of nondiabetic origin is seen in a variety of disorders and also produces glycosuria. Many of these disorders are associated with hormonal function and include pancreatitis, pancreatic cancer, acromegaly, Cushing syndrome, hyperthyroidism, and pheochromocytoma. The hormones glucagon, epinephrine, cortisol, thyroxine, and growth hormone, which are increased in these disorders, work in opposition to insulin, thereby producing hyperglycemia and glucosuria. Whereas a primary function of insulin is to convert glucose to glycogen for storage (glycogenesis), these opposing hormones cause the breakdown of glycogen to glucose (glycogenolysis), resulting in increased levels of circulating glucose. Epinephrine is also a strong inhibitor of insulin secretion and is increased when the body is subjected to severe stress, which accounts for the glucosuria seen in conjunction with cerebrovascular trauma and myocardial infarction.

Glycosuria occurs in the absence of hyperglycemia when the reabsorption of glucose by the renal tubules is compromised. This is frequently referred to as "renal glycosuria" and is seen in end-stage renal disease, cystinosis, and Fanconi syndrome. Glycosuria not associated with gestational diabetes is occasionally seen as a result of a temporary lowering of the renal threshold for glucose during pregnancy.

Reagent Strip (Glucose Oxidase) Reactions

Two very different tests have been used by laboratories to measure urinary glucose. The glucose oxidase procedure provides a specific test for glucose, and the copper reduction test is a general test for glucose and other reducing substances. Reagent strips employ the glucose oxidase testing method by impregnating the testing area with a mixture of glucose oxidase, peroxidase, chromogen, and buffer to produce a double sequential enzyme reaction. In the first step, glucose oxidase catalyzes a reaction between glucose and room air to produce gluconic acid and peroxide. In the second step, peroxidase catalyzes the reaction between peroxide and chromogen to form an oxidized colored compound that represents the presence of glucose.

1. Glucose +
$$O_2$$
 (air) $\xrightarrow{\text{glucose}}$ gluconic acid + H_2O_2

2.
$$H_2O_2$$
 + chromogen $\xrightarrow{peroxidase}$ oxidized colored chromogen + H_2O

Reagent strip manufacturers use several different chromogens, including potassium iodide (green to brown) and tetramethylbenzidine (yellow to green). Urine glucose may be

Summary of Glucose Reagent Strip Reagents Multistix: Glucose oxidase Peroxidase Potassium iodide Chemstrip: Glucose oxidase Peroxidase Tetramethylbenzidine Multistix: 75-125 mg/dL Sensitivity Chemstrip: 40 mg/dL Interference False-positive: Contamination by oxidizing agents and detergents False-negative: High levels of ascorbic acid High levels of ketones High specific gravity Low temperatures Improperly preserved

reported in terms of negative, trace, 1+, 2+, 3+, and 4+; however, the color charts also provide quantitative measurements ranging from 100 mg/dL to 2 g/dL, or 0.1% to 2%. The American Diabetes Association recommends quantitative reporting.

specimens

Ketones

Protein

Reaction Interference

Correlations

with other tests

Because the glucose oxidase method is specific for glucose, false-positive reactions are not obtained from other urinary constituents, including other sugars that may be present. False-positive reactions may occur, however, if containers become contaminated with peroxide or strong oxidizing detergents.

Substances that interfere with the enzymatic reaction or strong reducing agents, such as ascorbic acid, that prevent oxidation of the chromogen may produce false-negative results. To minimize interference from ascorbic acid, reagent strip manufacturers are incorporating additional chemicals into the test pads. An example is iodate that oxidizes ascorbic acid so that it cannot interfere with the oxidation of the chromogen. Product literature should be carefully reviewed for current information regarding all interfering substances. High levels of ketones also affect glucose oxidase tests at low glucose concentrations; however, because ketones are usually accompanied by marked glycosuria, this seldom presents a problem. High specific gravity and low temperature may decrease the sensitivity of the test. By far the greatest source of false-negative glucose results is the technical error of allow-

ing specimens to remain unpreserved at room temperature for extended periods. False-negative results are obtained with both the glucose oxidase and the copper reduction methods owing to the rapid glycolysis of glucose.

Copper Reduction Test

Measurement of glucose by the copper reduction method was one of the earliest chemical tests performed on urine. The test relies on the ability of glucose and other substances to reduce copper sulfate to cuprous oxide in the presence of alkali and heat. A color change progressing from a negative blue (CuSO_4) through green, yellow, and orange/red (Cu_2O) occurs when the reaction takes place.

$$CuSO_4$$
 (cupric sulfide) + reducing substance $\frac{heat}{alkali}$

$$\text{Cu}_2\text{O}$$
 (cuprous oxide) + oxidized substance \rightarrow color (blue/green \rightarrow orange/red)

The classic Benedict solution was developed in 1908 and contained copper sulfate, sodium carbonate, and sodium citrate buffer. 10 Urine was added to the solution, heat was applied, and the resulting precipitate was observed for color. A more convenient method that employs Benedict's principle is the Clinitest tablet (Siemens Medical Solutions Diagnostics, Tarrytown, N.Y.). The tablets contain copper sulfate, sodium carbonate, sodium citrate, and sodium hydroxide. Upon addition of the tablet to water and urine, heat is produced by the hydrolysis of sodium hydroxide and its reaction with sodium citrate, and carbon dioxide is released from the sodium carbonate to prevent room air from interfering with the reduction reaction. Tubes should be placed in a rack and not held in the hand because the reaction heat could cause a burn. At the conclusion of the effervescent reaction, the tube is gently shaken, and the color ranging from blue to orange/red can be compared with the manufacturer's color chart to determine the approximate amount of reducing substance.

Care must be taken to observe the reaction closely as it is taking place, because at high glucose levels, a phenomenon known as "pass through" may occur. When this happens, the color produced passes through the orange/red stage and returns to a green-brown color, and if not observed, a high glucose level may be reported as negative. An alternate method using two drops instead of five drops of urine can minimize the occurrence of "pass through." A separate color chart must be used to interpret the reaction. This chart provides values up to 5 g/dL, whereas the five-drop method is limited to 2 g/dL.

The sensitivity of Clinitest to glucose is reduced to a minimum of 200 mg/dL. As a nonspecific test for reducing substances, Clinitest is subject to interference from other reducing sugars, including galactose, lactose, fructose, maltose, pentoses, ascorbic acid, certain drug metabolites, and antibiotics such as the cephalosporins. Therefore, Clinitest does not provide a confirmatory test for glucose.

Clinitest tablets are very hygroscopic and should be stored in their tightly closed packages. A strong blue color in the unused tablets suggests deterioration due to moisture accumulation, as does vigorous tablet fizzing.

Comparison of Glucose Oxidase and Clinitest

There are several explanations for the finding of conflicting results between the two glucose tests. As stated, the Clinitest is not as sensitive as the glucose oxidase test, so the finding of a 1+ reagent strip reading and a negative Clinitest should not be surprising. A strongly positive reagent strip and a negative Clinitest, however, should cause concern about possible contamination by strong oxidizing agents. The most significant discrepancy is the negative reagent strip with a positive Clinitest. Although interfering substances affecting either test may cause this problem, the most frequent cause is the presence of other reducing sugars in the urine. Commonly found reducing sugars include galactose, fructose, pentose, and lactose, of which galactose is the most clinically significant. Galactose in the urine of a newborn represents an "inborn error of metabolism" in which lack of the enzyme galactose-1-phosphate uridyl transferase prevents breakdown of ingested galactose and results in failure to thrive and other complications, including death. All newborns should be screened for galactosuria because early detection followed by dietary restriction control the condition. Depending on the laboratory population, Clinitest is often performed on pediatric specimens from patients up to at least the age of 2 years. Many states have incorporated screening for galactosemia into their required newborn screening programs (see Chapter 9). The appearance of other reducing sugars is usually of minimal clinical significance, and lactose is frequently found in the urine of nursing mothers. Keep in mind that table sugar is sucrose, a nonreducing sugar, and does not react with Clinitest or glucose oxidase strips.

PROCEDURE



Clinitest Procedure

Place a glass test tube in a rack, add 5 drops of urine. Add 10 drops of distilled water to the urine in the test tube.

Drop one Clinitest tablet into the test tube and observe the reaction until completion (cessation of boiling).

CAUTION: The reaction mixture gets very hot. Do not touch the bottom area of the test tube. Use glass test tube only.

Wait 15 seconds after boiling has stopped and gently shake the contents of the tube.

Compare the color of the mixture to the Clinitest color chart and record the result in mg/dL or percent.

Observe for the possibility of the "pass-through" phenomenon.

Repeat using the 2-drop procedure.

Summary of Glucose Oxidase and Clinitest Reactions

Glucose Oxidase	Clinitest	Interpretation
I + positive	Negative	Small amount of glucose present
4+ positive	Negative	Possible oxidizing agent interference on reagent strip
Negative	Positive	Nonglucose reducing substance present Possible interfering sub- stance for reagent strip

Ketones

The term ketones represents three intermediate products of fat metabolism, namely, acetone, acetoacetic acid, and betahydroxybutyric acid. Normally, measurable amounts of ketones do not appear in the urine, because all the metabolized fat is completely broken down into carbon dioxide and water. However, when the use of available carbohydrate as the major source of energy becomes compromised, body stores of fat must be metabolized to supply energy. Ketones are then detected in urine.

Clinical Significance

Clinical reasons for increased fat metabolism include the inability to metabolize carbohydrate, as occurs in diabetes mellitus; increased loss of carbohydrate from vomiting; and inadequate intake of carbohydrate associated with starvation and malabsorption.

Testing for urinary ketones is most valuable in the management and monitoring of insulin-dependent (type 1) diabetes mellitus. *Ketonuria* shows a deficiency in insulin, indicating the need to regulate dosage. It is often an early indicator of insufficient insulin dosage in type 1 diabetes and in patients with diabetes who experience medical problems in

Clinical Significance of Urine Ketones

- I. Diabetic acidosis
- 2. Insulin dosage monitoring
- 3. Starvation
- 4. Malabsorption/pancreatic disorders
- 5. Strenuous exercise
- 6. Vomiting
- 7. Inborn errors of amino acid metabolism (see Chapter 9)

addition to diabetes. Increased accumulation of ketones in the blood leads to electrolyte imbalance, dehydration, and, if not corrected, acidosis and eventual diabetic coma. To aid in the monitoring of diabetes, ketone tests are not only included in all multiple-test strips, but are also combined with glucose on strips used primarily for at-home testing by patients diagnosed with diabetes.

The use of multiple-test strips in hospital laboratories often produces positive ketone tests unrelated to diabetes because the patient's illness either prevents adequate intake or absorption of carbohydrates or produces an accelerated loss, as in the case of vomiting. Weight-loss and eating disorder clinics can use a practical application of ketonuria produced by avoidance of carbohydrates to monitor patients. Frequent strenuous exercise can cause overuse of available carbohydrates and produce ketonuria.

Reagent Strip Reactions

The three ketone compounds are not present in equal amounts in urine. Both acetone and beta-hydroxybutyric acid are produced from acetoacetic acid (Fig. 5-1). The proportions of 78% beta-hydroxybutyric acid, 20% acetoacetic acid, and 2% acetone are relatively constant in all specimens.

Reagent strip tests use the sodium nitroprusside (nitroferricyanide) reaction to measure ketones. In this reaction, acetoacetic acid in an alkaline medium reacts with sodium nitroprusside to produce a purple color. The test does not measure beta-hydroxybutyric acid and is only slightly sensitive to acetone when glycine is also present; however, inasmuch as these compounds are derived from acetoacetic acid, their presence can be assumed, and it is not necessary to perform individual tests. Results are reported qualitatively as negative, trace, small (1+), moderate (2+), or large (3+), or semi-quantitatively as negative, trace (5 mg/dL), small (15 mg/dL), moderate (40 mg/dL), or large (80 to 160 mg/dL).

acetoacetate + sodium nitroprusside + (glycine) $\xrightarrow{\text{alkaline}}$ (and acetone) purple color

Acetest Tablets

In cases of severe ketosis, it may be necessary to perform tests on serial dilutions to provide more information as to the extent of ketosis. These are performed using a tablet test.

Acetest (Siemens Medical Solutions Diganostics, Tarrytown, N.Y.) provides sodium nitroprusside, glycine, disodium phosphate, and lactose in tablet form. The addition of lactose gives better color differentiation. An advantage of the Acetest

PROCEDURE



Acetest Procedure

Remove the Acetest tablet from the bottle and place on a clean dry piece of white paper.

Place one drop of urine on top of the tablet.

Wait 30 seconds.

Compare the tablet color with the manufacturersupplied color chart.

Report as negative, small, moderate, or large.

tablets is that they can also be used for serum and other body fluid testing. Acetest tablets are hygroscopic; if the specimen is not completely absorbed within 30 seconds, a new tablet should be used.

Reaction Interference

Specimens obtained following diagnostic procedures using the dyes phenolsulfonphthalein and bromsulphalein may produce an interfering red color in the alkaline test medium, as does highly pigmented red urine. Large amounts of levodopa and medications containing sulfhydryl groups, including mercaptoethane sulfonate sodium (MESNA) and captopril, may produce atypical color reactions. Reactions with interfering substances frequently fade on standing, whereas color development from acetoacetic acid increases, resulting in false-positive results from improperly timed readings. Falsely decreased values due to the volatilization of acetone and the breakdown of acetoacetic acid by bacteria are seen in improperly preserved specimens.

Blood

Blood may be present in the urine either in the form of intact red blood cells (*hematuria*) or as the product of red blood cell destruction, hemoglobin (*hemoglobinuria*). As discussed in Chapter 4, blood present in large quantities can be detected visually; hematuria produces a cloudy red urine, and hemoglobinuria appears as a clear red specimen. Because any amount of blood greater than five cells per microliter of urine is considered clinically significant, visual examination cannot be relied upon to detect the presence of blood. Microscopic examination of the urinary sediment shows intact red blood cells, but free hemoglobin produced either by hemolytic disorders or lysis of red blood cells is not detected.

Figure 5–I Production of acetone and betahydroxybutyric acid from acetoacetic acid.

Summary of Ketone Reagent Strip

Reagents Sodium nitroprusside

Glycine (Chemstrip)

Sensitivity Multistix: 5–10 mg/dL acetoacetic

acid

Chemstrip: 9 mg/dL acetoacetic acid;

70 mg/dL acetone

Interference False-positive: Phthalein dyes

Highly pigmented red urine

Levodopa

Medications containing free

sulfhydryl groups

False-negative: Improperly preserved speci-

mens

Correlations Glucose

with other tests

Therefore, chemical tests for hemoglobin provide the most accurate means for determining the presence of blood. Once blood has been detected, the microscopic examination can be used to differentiate between hematuria and hemoglobinuria.

Clinical Significance

The finding of a positive reagent strip test result for blood indicates the presence of red blood cells, hemoglobin, or myoglobin. Each of these has a different clinical significance.

Hematuria

Hematuria is most closely related to disorders of renal or genitourinary origin in which bleeding is the result of trauma or damage to the organs of these systems. Major causes of hematuria include renal calculi, glomerular diseases, tumors, trauma, pyelonephritis, exposure to toxic chemicals, and anticoagulant therapy. The laboratory is frequently requested to perform a urinalysis when patients presenting with severe back and abdominal pain are suspected of having renal calculi. In such cases, hematuria is usually of a small to moderate degree, but its presence can be essential to the diagnosis. Hematuria of nonpathologic significance is observed following strenuous exercise and during menstruation.

Hemoglobinuria

Hemoglobinuria may result from the lysis of red blood cells produced in the urinary tract, particularly in dilute, alkaline urine. It also may result from intravascular hemolysis and the subsequent filtering of hemoglobin through the glomerulus. Lysis of red blood cells in the urine usually shows a mixture of hemoglobinuria and hematuria, whereas no red blood cells are seen in cases of intravascular hemolysis. Under normal conditions, the formation of large hemoglobin-haptoglobin complexes in the circulation prevents the glomerular filtration of hemoglobin. When the amount of free hemoglobin present exceeds the haptoglobin content—as occurs in hemolytic anemias, transfusion reactions, severe burns, brown recluse spider bites, infections, and strenuous exercise—hemoglobin is available for glomerular filtration. Reabsorption of filtered hemoglobin also results in the appearance of large yellow-brown granules of denatured ferritin called hemosiderin in the renal tubular epithelial cells and in the urine sediment.

Myoglobinuria

Myoglobin, a heme-containing protein found in muscle tissue, not only reacts positively with the reagent strip test for blood but also produces a clear red-brown urine. The presence of myoglobin rather than hemoglobin should be suspected in patients with conditions associated with muscle destruction (*rhabdomyolysis*). Examples of these conditions include trauma, crush syndromes, prolonged coma, convulsions, muscle-wasting diseases, alcoholism, heroin abuse, and extensive exertion. The development of rhabdomylosis has been found to be a side effect in certain patients taking the cholesterol-lowering statin medications. ¹¹ The heme portion of myoglobin is toxic to the renal tubules, and high concentrations can cause acute renal failure. The massive hemoglobinuria seen in hemolytic transfusion reactions also is associated with acute renal failure.

Summary of Clinical Significance of a Positive Reaction for Blood

Hematuria

- I. Renal calculi
- 2. Glomerulonephritis
- 3. Pyelonephritis
- 4. Tumors
- 5. Trauma
- 6. Exposure to toxic chemicals
- 7. Anticoagulants
- 8. Strenuous exercise

Hemoglobinuria

- 1. Transfusion reactions
- 2. Hemolytic anemias
- 3. Severe burns
- 4. Infections/malaria

- 5. Strenuous exercise/red blood cell trauma
- 6. Brown recluse spider bites

Myoglobinuria

- I. Muscular trauma/crush syndromes
- 2. Prolonged coma
- 3. Convulsions
- 4. Muscle-wasting diseases
- 5. Alcoholism/overdose
- 6. Drug abuse
- 7. Extensive exertion
- 8. Cholesterol-lowering statin medications

Hemoglobinuria Versus Myoglobinuria

Occasionally the laboratory may be requested to differentiate between the presence of hemoglobin and myoglobin in a urine specimen. The diagnosis of *myoglobinuria* is usually based on the patient's history and elevated serum levels of the enzymes creatinine kinase and lactic dehydrogenase. The appearance of the patient's plasma can also aid in the differentiation. The kidneys rapidly clear myoglobin from the plasma, leaving a normal-appearing plasma, whereas hemoglobin bound to haptoglobin remains in the plasma and imparts a red color.

The concentration of myoglobin in the urine must be at least 25 mg/dL before the red pigmentation can be visualized. At this concentration, a precipitation test can be used to screen for the presence of myoglobin; 2.8 g of ammonium sulfate are added to 5 mL of centrifuged urine. After mixing and allowing the specimen to sit for 5 minutes, the urine is filtered or centrifuged, and the supernatant is tested for a reaction for blood with a reagent strip. The principle of this screening test is based on the fact that the larger hemoglobin molecules are precipitated by the ammonium sulfate, and myoglobin remains in the supernatant. Therefore, when myoglobin is present, the supernatant retains the red color and gives a positive reagent strip test for blood. Conversely, hemoglobin produces a red precipitate and a supernatant that tests negative for blood. Myoglobin is not stable in acidic urine and, if denatured, may precipitate with the ammonium sulfate. Specimens that cannot be tested immediately should be neutralized and frozen. Immunoassay procedures are available to measure serum myoglobin levels.

Reagent Strip Reactions

Chemical tests for blood use the pseudoperoxidase activity of hemoglobin to catalyze a reaction between hydrogen peroxide and the chromogen tetramethylbenzidine to produce an oxidized chromogen, which has a green-blue color.

$$H_2O_2$$
 + chromogen $\xrightarrow{\text{hemoglobin}}$ oxidized chromogen + H_2O

Reagent strip manufacturers incorporate peroxide, tetramethylbenzidine, and buffer into the blood testing area. Two color charts are provided that correspond to the reactions that occur with hemoglobinuria, myoglobinuria, and hematuria. In the presence of free hemoglobin/myoglobin, uniform color ranging from a negative yellow through green to a strongly positive green-blue appear on the pad. In contrast, intact red blood cells are lysed when they come in contact with the pad, and the liberated hemoglobin produces an isolated reaction that results in a speckled pattern on the pad. Reagent strip tests can detect concentrations as low as five red blood cells per microliter; however, care must be taken when comparing these figures with the actual microscopic values, because the absorbent nature of the pad attracts more than 1 mL of urine. The terms trace, small, moderate, and large or trace, 1+, 2+ and 3+ are used for reporting.

Blood Reagent Strip Summary Multistix: Diisopropylbenzene dehydroper-Reagents oxide tetramethylbenzidine Chemstrip: dimethyldihydroperoxyhexane tetramethylbenzidine Sensitivity Multistix: 5-20 RBCs/mL, 0.015-0.062 mg/dL hemoglobin Chemstrip: 5 RBCs/mL, hemoglobin corresponding to 10 RBCs/mL Interference False-positive: Strong oxidizing agents Bacterial peroxidases Menstrual contamination False-negative: High specific gravity/ crenated cells Formalin Captopril High concentrations of nitrite Ascorbic acid >25 mg/dL Unmixed specimens Correlations Protein with other Microscopic tests

Reaction Interference

False-positive reactions owing to menstrual contamination may be seen. They also occur if strong oxidizing detergents are present in the specimen container. Vegetable peroxidase and bacterial enzymes, including an *Escherichia coli* peroxidase, may also cause false-positive reactions. Therefore, sediments containing bacteria should be checked closely for the presence of red blood cells.

Traditionally, ascorbic acid has been associated with false-negative reagent strip reactions for blood. Both Multistix and Chemstrip have modified their reagent strips to reduce this interference to very high levels (25 mg/dL) of ascorbic acid. Multistix uses a peroxide that is less subject to reduction by ascorbic acid, and Chemstrip overlays the reagent pad with an iodate-impregnated mesh that oxidizes the ascorbic acid prior to its reaching the reaction pad. False-negative reactions can result when urine with a high specific gravity contains crenated red blood cells that do not lyse when they come in contact with the reagent pad. Decreased reactivity may also be seen when formalin is used as a preservative or when the hypertension medication, captopril, or high concentrations of nitrite (greater than 10 mg/dL) are present. Red blood cells settle to the bottom of the specimen container, and failure to mix the specimen prior to testing causes a falsely decreased reading.

■■● Bilirubin

The appearance of bilirubin in the urine can provide an early indication of liver disease. It is often detected long before the development of *jaundice*.

Production of Bilirubin

Bilirubin, a highly pigmented yellow compound, is a degradation product of hemoglobin. Under normal conditions, the life span of red blood cells is approximately 120 days, at which time they are destroyed in the spleen and liver by the phagocytic cells of the reticuloendothelial system. The liberated hemoglobin is broken down into its component parts: iron, protein, and protoporphyrin. The body reuses the iron and protein, and the cells of the reticuloendothelial system convert the remaining protoporphyrin to bilirubin. The bilirubin is then released into the circulation, where it binds with albumin and is transported to the liver. At this point, the kidneys cannot excrete the circulating bilirubin because not only is it bound to albumin, but it is also water insoluble. In the liver, bilirubin is conjugated with glucuronic acid by the action of glucuronyl transferase to form water-soluble bilirubin diglucuronide (conjugated bilirubin). Usually, this conjugated bilirubin does not appear in the urine because it is passed directly from the liver into the bile duct and on to the intestine. In the intestine, intestinal bacteria reduce bilirubin to urobilinogens, which is then oxidized and excreted in the feces in the form of urobilin. In Figure 5-2, bilirubin metabolism is illustrated for reference with this section and the subsequent discussion of urobilinogen.

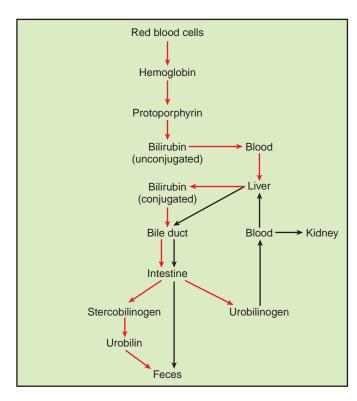


Figure 5–2 Hemoglobin degradation.

Clinical Significance

Conjugated bilirubin appears in the urine when the normal degradation cycle is disrupted by obstruction of the bile duct (e.g., gallstones or cancer) or when the integrity of the liver is damaged, allowing leakage of conjugated bilirubin into the circulation. Hepatitis and cirrhosis are common examples of conditions that produce liver damage, resulting in bilirubinuria. Not only does the detection of urinary bilirubin provide an early indication of liver disease, but also its presence or absence can be used in determining the cause of clinical jaundice. As shown in Table 5–3, this determination can be even more significant when bilirubin results are combined with urinary urobilinogen. Jaundice due to increased destruction of red blood cells does not produce bilirubinuria. This is because the serum bilirubin is present in the unconjugated form and the kidneys cannot excrete it.

Reagent Strip (Diazo) Reactions

Routine testing for urinary bilirubin by reagent strip uses the diazo reaction. Bilirubin combines with 2,4-dichloroaniline diazonium salt or 2,6-dichlorobenzene-diazonium-tetrafluoroborate in an acid medium to produce an azodye, with colors ranging from increasing degrees of tan or pink to violet, respectively. Qualitative results are reported as negative, small, moderate, or large, or as negative, 1+, 2+, or 3+. Reagent strip color reactions for bilirubin are more difficult to interpret than other reagent strip reactions and are easily influenced by other pigments present in the urine. Atypical color reactions are frequently noted on visual examination and are measured by automated readers. Further testing should be performed on any questionable results.¹²

bilirubin glucuronide + diazonium salt $\xrightarrow{\text{acid}}$ azodye

Ictotest Tablets

Questionable results can be repeated using the Ictotest (Siemen Medical Solutions Diagnostics, Tarrytown, N.Y.). The Ictotest is less subject to interference and is sensitive to 0.05 to 0.10 mg/dL of bilirubin, whereas the reagent strips have a lower sensitivity level of 0.40 mg/dL. Because

	Urine Bilirubin and Urobilinogen in Jaundice		
Urine Urine Bilirubin Urobilinogen			
Bile duct obstruction	+ + +	Normal	
Liver damage	+ or -	+ +	
Hemolytic disease	Negative	+ + +	

of its higher sensitivity, an Ictotest may be requested to detect early stages of liver disease. Ictotest kits consist of testing mats and tablets containing p-nitrobenzene-diazonium-ptoluenesulfonate, SSA, sodium carbonate, and boric acid. Ten drops of urine are added to the mat, which has special properties that cause bilirubin to remain on the surface as the urine is absorbed. Following the chemical reaction, a blue-to-purple color appears on the mat when bilirubin is present. Colors other than blue or purple appearing on the mat are considered to be a negative result. If interference in the Ictotest is suspected, it can usually be removed by adding water directly to the mat after the urine has been added. Interfering substances are washed into the mat, and only bilirubin remains on the surface. An Ictotest may be requested when early cases of liver disorders, such as hepatitis, are suspected.

Reaction Interference

As discussed previously, false-positive reactions are primarily due to urine pigments. Of particular concern are the yellow-orange urines from persons taking phenazopyridine compounds, because the thick pigment produced may be mistaken for bilirubin on initial examination. The presence of indican and metabolites of the medication Lodine may cause false-positive readings.

The false-negative results caused by the testing of specimens that are not fresh are the most frequent errors associated with bilirubin testing. Bilirubin is an unstable compound that is rapidly photo-oxidized to biliverdin when exposed to light. Biliverdin does not react with diazo tests. False-negative results also occur when hydrolysis of bilirubin diglucuronide produces free bilirubin, because this is less reactive in the reagent strip tests. High concentrations of ascorbic acid (greater than 25 mg/dL) and nitrite may lower the sensitivity of the test, because they combine with the diazonium salt and prevent its reaction with bilirubin.

Urobilinogen

As shown in Figure 5-2, when conjugated bilirubin is excreted through the bile duct into the intestine, the intestinal bacteria convert the bilirubin to a combination of urobilinogen and *stercobilinogen*. Some of the urobilinogen is reabsorbed from the intestine into the blood, recirculates to the liver, and is excreted back into the intestine through the

Summary of Clinical Significance of Urine Bilirubin

- I. Hepatitis
- 2. Cirrhosis
- 3. Other liver disorders
- 4. Biliary obstruction (gallstones, carcinoma)

PROCEDURE



Ictotest Procedure

Place 10 drops of urine onto one square of the absorbent test mat.

Using forceps, remove one Ictotest reagent tablet, recap the bottle promptly, and place the tablet in the center of the moistened area.

Place one drop of water onto the tablet and wait 5 seconds.

Place a second drop of water onto the tablet so that the water runs off the tablet onto the mat.

Observe the color of the mat around the tablet at the end of 60 seconds. The presence of a blue-to-purple color on the mat indicates that bilirubin is present. A slight pink or red color should be ignored. Report as positive or negative.

bile duct. The stercobilinogen cannot be reabsorbed and remains in the intestine where it is oxidized to urobilin and excreted in the feces. Urobilin is the pigment responsible for the characteristic brown color of feces. Urobilinogen appears in the urine because, as it circulates in the blood en route to the liver, it passes through the kidney and is filtered by the glomerulus. Therefore, a small amount of urobilinogen—less than 1 mg/dL or Ehrlich unit—is normally found in the urine.

Bilirubin	Reagent Strip Summary	
Reagents	Multistix: 2,4-dichloroaniline diazonium salt Chemstrip: 2,6-dichlorobenzene-diazonium salt	
Sensitivity	Multistix: 0.4–0.8 mg/dL bilirubin Chemstrip: 0.5 mg/dL bilirubin	
Interference	False-positive: Highly pigmented urines, phenazopyridine Indican (intestinal disorders) Metabolites of Lodine	
	False-negative: Specimen exposure to light Ascorbic acid >25 mg/dL High concentrations of nitrite	
Correlations with other tests	Urobilinogen	

70

Summary of Clinical Significance of Urine Urobilinogen

- 1. Early detection of liver disease
- 2. Liver disorders, hepatitis, cirrhosis, carcinoma
- 3. Hemolytic disorders

Clinical Significance

Increased urine urobilinogen (greater than 1 mg/dL) is seen in liver disease and hemolytic disorders. Measurement of urine urobilinogen can be valuable in the detection of early liver disease; however, studies have shown that when urobilinogen tests are routinely performed, 1% of the non-hospitalized population and 9% of a hospitalized population exhibit elevated results. This is frequently caused by constipation.

Impairment of liver function decreases the ability of the liver to process the urobilinogen recirculated from the intestine. The excess urobilinogen remaining in the blood is filtered by the kidneys and appears in the urine.

The clinical jaundice associated with hemolytic disorders results from the increased amount of circulating unconjugated bilirubin. This unconjugated bilirubin is presented to the liver for conjugation, resulting in a markedly increased amount of conjugated bilirubin entering the intestines. As a result, increased urobilinogen is produced, and increased amounts of urobilinogen are reabsorbed into the blood and circulated through the kidneys where filtration takes place. In addition, the overworked liver does not process the reabsorbed urobilinogen as efficiently, and additional urobilinogen is presented for urinary excretion.

Although it cannot be determined by reagent strip, the absence of urobilinogen in the urine and feces is also diagnostically significant and represents an obstruction of the bile duct that prevents the normal passage of bilirubin into the intestine. An additional observation is the production of pale stools as the result of the lack of urobilin. See Table 5–3 for an outline of the relationship of urine urobilinogen and bilirubin to the pathologic conditions associated with them.

Reagent Strip Reactions and Interference

The reagent strip reactions for urobilinogen differ between Multistix and Chemstrip much more significantly than do other reagent strip parameters. Multistix uses Ehrlich's aldehyde reaction, in which urobilinogen reacts with *p*-dimethylaminobenzaldehyde (Ehrlich reagent) to produce colors ranging from light to dark pink. Results are reported as Ehrlich units (EU), which are equal to mg/dL, ranging from normal readings of 0.2 and 1 through abnormal readings of 2, 4, and 8. Chemstrip incorporates an azo-coupling (diazo) reaction using 4-methoxybenzene-diazonium-tetrafluoroborate to react with urobilinogen, producing colors ranging

from white to pink. This reaction is more specific for urobilinogen than the Ehrlich reaction. Results are reported in mg/dL. Both tests detect urobilinogen that is present in normal quantities, and color comparisons are provided for the upper limits of normal as well as abnormal concentrations. Reagent strip tests cannot determine the absence of urobilinogen, which is significant in biliary obstruction.

MULTISTIX:

```
urobilinogen + p-dimethylaminobenzaldehyde \xrightarrow{\text{acid}} red color (Ehrlich's (Ehrlich reagent) reactive substances)
```

CHEMSTRIP:

urobilinogen + diazonium salt → red azodye (4-methyloxybenzene-diazonium-tetrafluoroborate)

Reaction Interference

The Ehrlich reaction on Multistix is subject to a variety of interferences, referred to as Ehrlich-reactive compounds, that produce false-positive reactions. These include porphobilinogen, indican, *p*-aminosalicylic acid, sulfonamides, methyldopa, procaine, and chlorpromazine compounds. The presence of porphobilinogen is clinically significant; however, the reagent strip test is not considered a reliable method to screen for its presence. Porphobilinogen is discussed later in this section and in Chapter 9.

The sensitivity of the Ehrlich reaction increases with temperature, and testing should be performed at room temperature. Highly pigmented urines cause atypical readings with both brands of reagent strips. As a result of increased excretion of bile salts, urobilinogen results are normally highest following a heavy meal.

False-negative results occur most frequently when specimens are improperly preserved, allowing urobilinogen to be photo-oxidized to urobilin. High concentrations of nitrite interfere with the azo-coupling reaction on Chemstrip. False-negative readings also are obtained with both strips when formalin is used as a preservative.

Ehrlich Tube Test

Prior to the development of reagent strip methods, tests for urobilinogen were not performed routinely because the available procedures were time consuming and nonspecific. When clinically necessary, a tube test was performed using Ehrlich reagent. Addition of Ehrlich reagent to urine produces a cherry-red color. Addition of sodium acetate enhances the color reaction. In the tube method, one part Ehrlich reagent was added to 10 parts of urine. The tube was mixed and examined for a red color.

Unfortunately, as discussed previously, this test also was subjected to false-positive results when porphobilinogen and

Urobilinogen Reagent Strip Summary Multistix: p-dimethylaminobenzaldehyde Reagents Chemstrip: 4-methoxybenzene-diazoniumtetrafluoroborate Multistix: 0.2 mg/dL urobilinogen Sensitivity Chemstrip: 0.4 mg/dL urobilinogen Interference Multistix: False-positive: Porphobilinogen Indican p-aminosalicylic acid Sulfonamides Methyldopa Procaine Chlorpromazine Highly-pigmented urine False-negative: Old specimens Preservation in for-Chemstrip: False-positive: Highly-pigmented urine False-negative: Old specimens Preservation in formalin High concentrations of nitrate Correlations Bilirubin with other tests

Ehrlich-reactive compounds were present. As a result, the tube test was modified to differentiate among urobilinogen, porphobilinogen, and Ehrlich-reactive compounds.

Watson-Schwartz Differentiation Test

The classic test for differentiating between urobilinogen, porphobilinogen, and Ehrlich-reactive compounds is the Watson-Schwartz test. ¹⁴ The test is performed as follows:

Tube 1 Tube 2
2 mL urine 2 mL urine
2 mL chloroform 2 mL butanol
4 mL sodium acetate 4 mL sodium acetate

The addition of chloroform to Tube 1 results in the extraction of urobilinogen into the chloroform (bottom) layer, producing a colorless urine (top) layer, and a red chloroform layer on the bottom. Neither porphobilinogen nor other Ehrlich-reactive compounds are soluble in chloroform. Porphobilinogen is also not soluble in butanol; however, urobilinogen and other Ehrlich-reactive compounds are

PROCEDURE



Watson-Schwartz Test

Label 2 tubes #1 and #2
Tube 1 Tube 2
2 mL urine 2 mL urine
2 mL chloroform 2 mL butanol

4 mL sodium acetate 4 mL sodium acetate

Vigorously shake both tubes. Place in a rack for layers to settle.

Observe both tubes for red color in the layers.

Interpretation:

Tube 1

Upper layer = urine; if colorless = porphobilinogen or Ehrlich-reactive compounds.

Bottom layer = chloroform; if red = urobilinogen. If both layers are red re-extract the urine layer from tube 1.

Place 2 mL of urine layer from tube 1 and 2 mL chloroform and 4 mL sodium acetate into a new tube. Repeat procedure.

Interpretation: Upper layer – urine colorless

Bottom layer – chloroform—red = excess urobilinogen Both layers red = porphobilinogen and urobilinogen

Tube 2

Upper layer = butanol If red = urobilinogen or

Ehrlich-reactive compounds

 $Bottom\ layer = urine \qquad If\ colorless = porphobilinogen$

extracted into butanol. Therefore, the addition of butanol to Tube 2 produces a red (upper) butanol layer if urobilinogen or Ehrlich-reactive compounds are present and a colorless butanol layer if porphobilinogen is present. As shown in Figure 5-3, Table 5-4, and the Procedure Box, urobilinogen is soluble in both chloroform and butanol, and porphobilinogen is soluble in neither. If both urobilinogen and porphobilinogen are present, both layers appear red. Before reporting the test as positive for both substances, an additional chloroform extraction should be performed on the red urine (upper) layer in Tube 1 to ensure that the red color is not due to excess urobilinogen.

Hoesch Screening Test for Porphobilinogen

The Hoesch test is used for rapid screening or monitoring of urinary porphobilinogen. Two drops of urine are added to approximately 2 mL of Hoesch reagent (Ehrlich reagent dissolved in 6 M HCl), and the top of the solution is immediately observed for the appearance of a red color that indicates the presence of porphobilinogen. When the tube is shaken, the red color is seen throughout the solution. The test detects approximately 2 mg/dL of porphobilinogen, and urobilinogen is inhibited by the highly acidic pH. High concentrations of

72 CHAPTER 5 • Chemical Examination of Urine

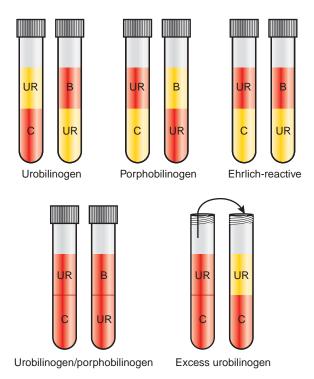


Figure 5-3 Typical Ehrlich reactions.

methyldopa and indican, and highly pigmented urines, may produce false-positive results.

■■● Nitrite

Clinical Significance

The reagent strip test for nitrite provides a rapid screening test for the presence of urinary tract infection (UTI). The test is designed to detect cases in which the need for a culture may not be apparent; it is not intended to replace the urine culture as the primary test for diagnosing and monitoring bacterial infection. Many UTIs are believed to start in the bladder as a result of external contamination and, if untreated, progress upward through the ureters to the tubules, renal pelvis, and kidney. The nitrite test is valuable for detecting initial bladder infection (cystitis), because patients are often asymptomatic or have vague symptoms that would not lead the physician to order a urine culture. Pyelonephritis, an inflammatory process of the kidney and adjacent renal pelvis, is a frequent complication of untreated cystitis and can lead to renal tissue damage, impairment of renal function, hypertension, and even septicemia. Therefore, detection of bacteriuria through the use of the nitrite screening test and subsequent antibiotic therapy can prevent these serious complications. The nitrite test also can be used to evaluate the success of antibiotic therapy and to periodically screen persons with recurrent infections, patients with diabetes, and pregnant women, all of whom are considered to be at high risk for UTI. 15 As discussed in the following section, many laboratories use the nitrite test in combination with the leukocyte esterase test to determine the necessity of performing urine cultures.

Table 5–4	Watson-Schwartz Test Interpretation		
	Ot Urobilinogen	her Ehrlich- Reactive Substances	Porpho- bilinogen
Chloroform Extr	action		
Urine (Top Layer)	Colorless	Red	Red
Chloroform (Bottom Layer)	Red	Colorless	Colorless
Butanol Extracti	on		
Butanol (Top Layer)	Red	Red	Colorless
Urine (Bottom Layer)	Colorless	Colorless	Red

Reagent Strip Reactions

The chemical basis of the nitrite test is the ability of certain bacteria to reduce nitrate, a normal constituent of urine, to nitrite, which does not normally appear in the urine. Nitrite is detected by the Greiss reaction, in which nitrite at an acidic pH reacts with an aromatic amine (para-arsanilic acid or sulfanilamide) to form a diazonium compound that then reacts with tetrahydrobenzoquinolin compounds to produce a pink-colored azodye. To prevent false-positive reactions in externally contaminated specimens, the sensitivity of the test is standardized to correspond with a quantitative bacterial culture criterion of 100,000 organisms per milliliter. Although different shades of pink may be produced, the test does not measure the degree of bacteriuria, and any shade of pink is considered to represent a clinically significant amount of bacteria. Results are reported only as negative or positive.

para-arsanilic acid or sulfanilamide + NO $_2 \xrightarrow{\text{acid}}$ diazonium salt (nitrite)

diazonium salt + tetrahydrobenzoquinolin $\xrightarrow{\text{acid}}$ pink azodye

Summary of Clinical Significance of Urine Nitrite

- 1. Cystitis
- 2. Pyelonephritis
- 3. Evaluation of antibiotic therapy
- 4. Monitoring of patients at high risk for urinary tract infection
- 5. Screening of urine culture specimens

Reaction Interference

Several major factors can influence the reliability of the nitrite test, and tests with negative results in the presence of even vaguely suspicious clinical symptoms should always be repeated or followed by a urine culture.

- 1. Bacteria that lack the enzyme reductase do not possess the ability to reduce nitrate to nitrite. Reductase is found in the gram-negative bacteria (Enterobacteriaceae) that most frequently cause UTIs. Non–nitrate-reducing gram-positive bacteria and yeasts, however, cause a significant number of infections, and the nitrite test does not detect the presence of these organisms.
- 2. Bacteria capable of reducing nitrate must remain in contact with the urinary nitrate long enough to produce nitrite. Therefore, nitrite tests should be performed on first morning specimens or specimens collected after urine has remained in the bladder for at least 4 hours. The correlation between positive cultures and positive nitrite test results is significantly lower when testing is performed on random samples.

Nitrite Reagent Strip Summary

Reagents Multistix: p-arsanilic acid Tetrahydrobenzo(h)-quinolin-3-ol Chemstrip: Sulfanilamide, hydroxytetrahydro benzoquinoline Multistix: 0.06-0.1 mg/dL nitrite ion Sensitivity Chemstrip: 0.05 mg/dL nitrite ion Interference False-negative: Nonreductase-containing bacteria Insufficient contact time between bacteria and urinary nitrate Lack of urinary nitrate Large quantities of bacteria converting nitrite to nitrogen Presence of antibiotics High concentrations of ascorbic acid High specific gravity False-positive: Improperly preserved spec-

imens

Highly pigmented urine

Correlations Protein
with other Leukocytes
tests Microscopic

- 3. The reliability of the test depends on the presence of adequate amounts of nitrate in the urine. This is seldom a problem in patients on a normal diet that contains green vegetables; however, because diet usually is not controlled prior to testing, the possibility of a false-negative result owing to lack of dietary nitrate does exist.
- 4. Further reduction of nitrite to nitrogen may occur when large numbers of bacteria are present, and this causes a false-negative reaction.
- 5. Other causes of false-negative results include inhibition of bacterial metabolism by the presence of antibiotics, large quantities of ascorbic acid interfering with the diazo reaction, and decreased sensitivity in specimens with a high specific gravity. Large amounts of ascorbic acid compete with nitrite to combine with the diazonium salt, therefore preventing a true nitrite measurement.

False-positive results are obtained if nitrite testing is not performed on fresh samples, because multiplication of contaminant bacteria soon produces measurable amounts of nitrite. A true positive nitrite test should be accompanied by a positive leukocyte esterase test. When fresh urine is used, false-positive results are not obtained, even if a nonsterile container is used. Pink discoloration or spotting on the edges of the reagent pad should not be considered a positive reaction. Highly pigmented urines produce atypical color reactions. Visual examination of the strip will determine that the characteristic pink color is not present. Automated strip readers report any color change as positive, and strips should be visually examined when discrepancies are observed.

Leukocyte Esterase

Prior to the development of the reagent strip leukocyte esterase (LE) test, detection of increased urinary leukocytes required microscopic examination of the urine sediment. This can be subject to variation depending on the method used to prepare the sediment and the technical personnel examining the sediment. Therefore, the chemical test for leukocytes offers a more standardized means for the detection of leukocytes. The test is not designed to measure the concentration of leukocytes, and the manufacturers recommend that quantitation be done by microscopic examination. An additional advantage to the chemical LE test is that it detects the presence of leukocytes that have been lysed, particularly in dilute alkaline urine, and would not appear in the microscopic examination.

Clinical Significance

Normal values for leukocytes are based on the microscopic sediment examination and vary from 0 to 2 to 0 to 5 per high-power field. Women tend to have higher numbers than men as a result of vaginal contamination. Increased urinary leukocytes are indicators of UTI. The LE test detects the presence

Summary of Clinical Significance of Urine Leukocytes

- I. Bacterial and nonbacterial urinary tract infection
- 2. Inflammation of the urinary tract
- 3. Screening of urine culture specimens

of esterase in the granulocytic white blood cells (neutrophils, eosinophils, and basophils) and monocytes. Neutrophils are the leukocytes most frequently associated with bacterial infections. Esterases also are present in Trichomonas and histiocytes. Lymphocytes, erythrocytes, bacteria, and renal tissue cells do not contain esterases. A positive LE test result is most frequently accompanied by the presence of bacteria, which, as discussed previously, may or may not produce a positive nitrite reaction. Infections caused by Trichomonas, Chlamydia, yeast, and inflammation of renal tissues (i.e., interstitial nephritis) produce leukocyturia without bacteriuria.

Screening urine specimens using the LE and nitrite chemical reactions to determine the necessity of performing urine cultures can be a cost-effective measure. 16 The LE test contributes significantly more to the reliability of this practice than does the nitrite test.

Reagent Strip Reaction

The reagent strip reaction uses the action of LE to catalyze the hydrolysis of an acid ester embedded on the reagent pad to produce an aromatic compound and acid. The aromatic compound then combines with a diazonium salt present on the pad to produce a purple azodye.

indoxylcarbonic acid ester
$$\xrightarrow[\text{esterases}]{\text{leukocyte}}$$
 indoxyl + acid indoxyl + diazonium salt $\xrightarrow[\text{acid}]{\text{acid}}$ purple azodye

The LE reaction requires the longest time of all the reagent strip reactions (2 minutes). Reactions are reported as trace, small, moderate, and large or trace, 1+, 2+, and 3+. Trace readings may not be significant and should be repeated on a fresh specimen.

Reaction Interference

The presence of strong oxidizing agents or formalin in the collection container causes false-positive reactions. Highly pigmented urines and the presence of nitrofurantoin obscure the color reaction.

False-negative results may occur in the presence of high concentrations of protein (greater than 500 mg/dL), glucose (greater than 3 g/dL), oxalic acid, and ascorbic acid. In this reaction, ascorbic acid also combines with the diazonium salt.

Leukocyte Esterase Reagent **Strip Summary**

Reagents Multistix: Derivatized pyrrole amino acid

ester

Diazonium salt

Chemstrip: Indoxylcarbonic acid

ester

Diazonium salt

Sensitivity Multistix: 5-15 WBC/hpf

Chemstrip: 10-25 WBC/hpf

Interference False-positive: Strong oxidizing agents

Formalin

Highly pigmented urine,

nitrofurantoin

False-negative: High concentrations of pro-

tein, glucose, oxalic acid, ascorbic acid, gentamicin, cephalosporins, tetracyclines, inaccurate timing

Correlations Protein with other Nitrite Microscopic tests

Crenation of leukocytes preventing release of esterases may occur in urines with a high specific gravity. 17 The presence of the antibiotics gentamicin, cephalexin, cephalothin, and tetracycline decreases the sensitivity of the reaction.

Specific Gravity

The addition of a specific gravity testing area to reagent strips has eliminated a time-consuming step in routine urinalysis and has provided a convenient method for routine screening. Replacing osmometry or refractometry for critical fluid monitoring is not recommended. 18 The clinical significance of the specific gravity test is discussed in Chapter 4.

Clinical Significance of **Urine Specific Gravity**

- I. Monitoring patient hydration and dehydration
- 2. Loss of renal tubular concentrating ability
- 3. Diabetes insipidus
- 4. Determination of unsatisfactory specimens due to low concentration

Reagent Strip Reaction

The reagent strip reaction is based on the change in pK_a (dissociation constant) of a polyelectrolyte in an alkaline medium. The polyelectrolyte ionizes, releasing hydrogen ions in proportion to the number of ions in the solution. The higher the concentration of urine, the more hydrogen ions are released, thereby lowering the pH. Incorporation of the indicator bromthymol blue on the reagent pad measures the change in pH. As the specific gravity increases, the indicator changes from blue (1.000 [alkaline]), through shades of green, to yellow (1.030 [acid]). Readings can be made in 0.005 intervals by careful comparison with the color chart. The specific gravity reaction is diagrammed in Figure 5-4.

Reaction Interference

The reagent strip specific gravity measures only ionic solutes, thereby eliminating the interference by the large organic molecules, such as urea and glucose, and by radiographic contrast media and plasma expanders that are included in physical measurements of specific gravity. This difference must be considered when comparing specific gravity results obtained by a different method. Elevated concentrations of protein slightly increase the readings as a result of protein anions.

Specimens with a pH of 6.5 or higher have decreased readings caused by interference with the bromthymol blue indicator (the blue-green readings associated with an alkaline pH correspond to a low specific gravity reading). Therefore, manufacturers recommend adding 0.005 to specific gravity

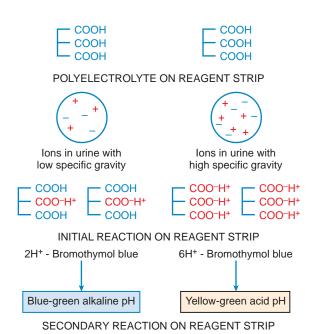


Figure 5-4 Diagram of reagent strip-specific gravity reaction.

Urine Specific Gravity Reagent Strip Summary

Reagents Multistix: Poly (methyl vinyl ether/maleic anhydride) bromthymol blue Chemstrip: Ethyleneglycoldiaminoethylethertetraacetic acid, bromthymol blue

Sensitivity 1.000–1.030

Interference False-positive: High concentrations of protein False-negative: Highly alkaline urines (>6.5)

readings when the pH is 6.5 or higher. The correction is performed by automated strip readers.

References

- 1. TechniTips, Miles Diagnostics, Elkhart, Ind. October, 1992.
- 2. Renfrew, G: Confirmatory testing in urinalysis. Urinalysis News 10(3), 1994.
- 3. Pugia, MJ, and Lott, JA: New developments in urinalysis strip tests for proteins. In Bayer Encylopedia of Urinalysis. Bayer Diagnostics, Elkhart, Ind., 2002.
- 4. Bhuwnesh, A, et al: Microalbumin screening by reagent strip predicts cardiovascular risk in hypertension. J Hypertens 14: 223-228, 1992.
- 5. Bianchi, S, et al: Microalbuminurea in essential hypertension. J Nephrol 10(4):216-219, 1997.
- Abuela, G: Proteinuria: Diagnostic principles and procedures. Ann Intern Med 98:1986-1991, 1983.
- 7. Clinitek Microalbumin Reagent Strip Product Insert. Bayer Diagnostics, Elkhart, Ind., 2006.
- 8. Multistix Pro Reagent Strips Product Insert. Bayer Diagnostics, Elkhart, Ind., 2005.
- 9. Guthrie, D, Hinnen, D, and Guthrie, R: Single-voided vs. double-voided urine testing. Diabetes Care 2(3):269-271, 1979.
- 10. Benedict, SR: A reagent for the detection of reducing sugars. J Biol Chem 5:485-487, 1909.
- 11. Lane R, Phillips, M: rhabdomylosis has many causes including statins and may be fatal. Brit J Med 327:115-116, 2003.
- 12. Riddhimat, R, Hiranras, S, and Petchclair, B: Retesting atypical reactions in urine bilirubin tests. Clin Lab Sci 5(5):310-312, 1007
- 13. Hager, CB, and Free, AH: Urine urobilinogen as a component of routine urinalysis. Am J Med Technol 36(5):227-233, 1970.
- 14. Watson, CJ, and Schwartz, S: A simple test for urinary porphobilinogen. Proc Soc Exp Biol Med 47:393-394, 1941.
- 15. Kunin, CM, and DeGroot, JE: Self-screening for significant bacteriuria. JAMA 231(13):1349-1353, 1975.
- 16. Wise, KA, Sagert, LA, and Grammens, GL: Urine leukocyte esterase and nitrite tests as an aid to predict urine culture results. Lab Med 15(3):186-187, 1984.
- 17. Scheer, WD: The detection of leukocyte esterase activity in urine with a new reagent strip. Am J Clin Pathol 87(1):86-93, 1987.
- 18. Romolo, D, et al: Refractometry, test strip and osmometery compared as measures of relative density of urine. Clin Chem 33(1):190, 1987.

©2008 F. A. Davis

STUDY

 Leaving a reagent strip in the specimen for too long will:

CHAPTER 5 • Chemical Examination of Urine

- A. Cause runover between reagent pads
- B. Alter the color of the specimen
- C. Cause reagents to leach from the pads
- D. Not affect the chemical reactions
- **2.** Failure to mix a specimen prior to inserting the reagent strip will primarily affect the:
 - A. Glucose reading
 - B. Blood reading
 - C. Nitrite reading
 - D. Ph reading
- **3.** Testing a refrigerated specimen that has not warmed to room temperature will adversely affect:
 - A. Enzymatic reactions
 - B. Dye-binding reactions
 - C. The sodium nitroprusside reaction
 - D. Diazo reactions
- **4.** The reagent strip reaction that requires the longest reaction time is the:
 - A. Bilirubin
 - В. рН
 - C. Leukocyte esterase
 - D. Glucose
- **5.** Quality control of reagent strips is performed:
 - A. Using positive and negative controls
 - B. When results are questionable
 - C. At least once every 24 hours
 - D. All of the above
- **6.** All of the following are important to protect the integrity of reagent strips *except*:
 - A. Removing the desiccant from the bottle
 - B. Storing in an opaque bottle
 - C. Storing at room temperature
 - D. Resealing the bottle after removing a strip
- 7. The principle of the reagent strip test for pH is the:
 - A. Protein error of indicators
 - B. Greiss reaction
 - C. Dissociation of a polyelectrolyte
 - D. Double indicator reaction
- **8.** A urine specimen with a pH of 9.0:
 - A. Is indicative of metabolic acidosis
 - B. Should be recollected
 - C. May contain calcium oxalate crystals
 - D. Is seen after drinking cranberry juice

- **9.** In the laboratory, a primary consideration associated with pH is:
 - A. Identification of urinary crystals
 - B. Monitoring of vegetarian diets
 - C. Determination of specimen acceptability
 - D. Both A and C
- **10.** Indicate the source of the following proteinurias by placing a 1 for prerenal, 2 for renal, or 3 for postrenal in front of the condition.
 - A. ____Microalbuminuria
 - B. ____Acute phase reactants
 - C. ____Pre-eclampsia
 - D. ____Vaginal inflammation
 - E. ____Multiple myeloma
 - F. ___Orthostatic proteinuria
 - G. ____Prostatitis
- **11**. The principle of the protein error of indicators reaction is that:
 - A. Protein changes the pH of the urine
 - B. Albumin accepts hydrogen ions from the indicator
 - C. The indicator accepts ions for albumin
 - D. Albumin changes the pH of the urine
- **12**. All of the following will cause false-positive protein reagent strip values *except*:
 - A. Proteins other than albumin
 - B. Highly buffered alkaline urines
 - C. Delay in removing the reagent strip from the specimen
 - D. Contamination by quartenary ammonium compounds
- **13**. A patient with a 1+ protein reading in the afternoon is asked to submit a first morning specimen. The second specimen also has a 1+ protein. This patient is:
 - A. Positive for orthostatic proteinuria
 - B. Negative for orthostatic proteinuria
 - C. Positive for Bence Jones protein
 - D. Negative for clinical proteinuria
- **14.** Testing for microalbuminuria is valuable for monitoring patients with:
 - A. Hypertension
 - B. Diabetes mellitus
 - C. Cardiovascular disease risk
 - D. All of the above
- **15**. All of the following are true for the Micral-Test for microalbumin *except*:
 - A. It is run on first morning specimens
 - B. It contains an antibody-enzyme conjugate
 - C. Two blue bands are formed on the strip
 - D. Unbound antibody attaches to immobilized albumin

- **16.** All of the following are true for the Immunodip test for microalbumin *except*:
 - A. Unbound antibody migrates farther than bound antibody
 - B. Blue latex particles are coated with antibody
 - C. Bound antibody migrates further than unbound antibody
 - D. It utilizes an immumochromographic principle
- **17**. The principle of the protein-low reagent pad on the Multistix Pro is the:
 - A. Binding of albumin to sulphonphtalein dye
 - B. Immunologic binding of albumin to antibody
 - C. Reverse protein error of indicators reaction
 - D. Enzymatic reaction between albumin and dye
- **18**. The principle of the creatinine reagent pad on microalbumin reagent strips is the:
 - A. Double indicator reaction
 - B. Diazo reaction
 - C. Pseudoperoxidase reaction
 - D. Reduction of a chromogen
- **19**. The purpose of performing an albumin:creatinine ratio is to:
 - A. Estimate the glomerular filtration rate
 - B. Correct for hydration in random specimens
 - C. Avoid interference for alkaline urines
 - D. Correct for abnormally colored urines
- **20**. A patient with a normal blood glucose and a positive urine glucose should be further checked for:
 - A. Diabetes mellitus
 - B. Renal disease
 - C. Gestational diabetes
 - D. Pancreatitis
- **21.** The principle of the reagent strip tests for glucose is the:
 - A. Peroxidase activity of glucose
 - B. Glucose oxidase reaction
 - C. Double sequential enzyme reaction
 - D. Dye-binding of glucose and chromogen
- **22.** All of the following may produce false-negative glucose reactions *except*:
 - A. Detergent contamination
 - B. Ascorbic acid
 - C. Unpreserved specimens
 - D. Low urine temperature
- **23.** A positive Clinitest and a negative reagent strip glucose are indicative of:
 - A. Low levels of glucose
 - B. Nonglucose reducing substances
 - C. High levels of glucose
 - D. Both A and B

- **24**. The primary reason for performing a Clinitest is to:
 - A. Check for high ascorbic acid levels
 - B. Confirm a positive reagent strip glucose
 - C. Check for newborn galactosuria
 - D. Confirm a negative glucose reading
- **25**. The three intermediate products of fat metabolism include all of the following *except*:
 - A. Acetoacetic acid
 - B. Ketoacetic acid
 - C. Beta-hydroxybutyric acid
 - D. Acetone
- **26**. The most significant reagent strip test that is associated with a positive ketone result is:
 - A. Glucose
 - B. Protein
 - C. pH
 - D. Specific gravity
- **27**. The primary reagent in the reagent strip test for ketones is:
 - A. Glycine
 - B. Lactose
 - C. Sodium hydroxide
 - D. Sodium nitroprusside
- **28.** Ketonuria may be caused by all of the following *except:*
 - A. Bacterial infections
 - B. Diabetic acidosis
 - C. Starvation
 - D. Vomiting
- **29**. Urinalysis on a patient with severe back and abdominal pain is frequently performed to check for:
 - A. Glucosuria
 - B. Proteinuria
 - C. Hematuria
 - D. Hemoglobinuria
- **30.** Place the appropriate number or numbers in front of each of the following statements. Use both numbers for an answer if needed.
 - 1. Hemoglobinuria
 - 2. Myoglobinuria
 - A. ____Associated with transfusion reactions
 - B. ____Clear, red urine and pale yellow plasma
 - C. ____Clear, red urine and red plasma
 - D. ____Associated with rhabdomylosis
 - E. ____Precipitated by ammonium sulfate
 - F. ____Not precipitated by ammonium sulfate
 - G. ____Produced hemosiderin granules in urinary sediments
 - H. Associated with acute renal failure

78 CHAPTER 5 • Chemical Examination of Urine

Continued

©2008 F. A. Davis

- **31.** The principle of the reagent strip test for blood is based on the:
 - A. Binding of heme and a chromogenic dye
 - B. Peroxidase activity of heme
 - C. Reaction of peroxide and chromogen
 - D. Diazo activity of heme
- **32.** A speckled pattern on the blood pad of the reagent strip indicates:
 - A. Hematuria
 - B. Hemoglobinuria
 - C. Myoglobinuria
 - D. All of the above
- **33.** List the following products of hemoglobin degradation in the correct order by placing numbers 1–4 in front of them.
 - A. ___Conjugated bilirubin
 - B. ___Urobilinogen and stercobiligen
 - C. ___Urobilin
 - D. ___Unconjugated bilirubin
- **34.** The principle of the reagent strip test for bilirubin is the:
 - A. Diazo reaction
 - B. Ehrlich reaction
 - C. Greiss reaction
 - D. Peroxidase reaction
- **35**. An elevated urine bilirubin with a normal urobilinogen is indicative of:
 - A. Cirrhosis of the liver
 - B. Hemolytic disease
 - C. Hepatitis
 - D. Biliary obstruction
- **36.** The primary cause of a false-negative bilirubin reaction is:
 - A. Highly pigmented urine
 - B. Specimen contamination
 - C. Specimen exposure to light
 - D. Excess conjugated bilirubin
- **37**. The purpose of the special mat supplied with the Ictotest tablets is that:
 - A. Bilirubin remains on the surface of the mat.
 - B. It contains the dye needed to produce color.
 - C. It removes interfering substances.
 - D. Bilirubin is absorbed into the mat.
- **38.** The reagent in the Multistix reaction for urobilinogen is:
 - A. A diazonium salt
 - B. Tetramethylbenzidine
 - C. p-dimethylaminobenzaldehyde
 - D. Hoesch reagent

- **39.** The primary problem with urobilinogen tests using Ehrlich reagent is:
 - A. Positive reactions with porphobilinogen
 - B. Lack of sensitivity
 - C. Positive reactions with Ehrlich reactive substances
 - D. Both A and C
- **40**. In the Watson-Schwartz differentiation test, the substance(s) not extracted into butanol is/are:
 - A. Urobilinogen
 - B. Porphobilinogen
 - C. Ehrlich reactive substances
 - D. All of the above
- **41.** The Hoesch test is used to monitor or screen patients for the presence of:
 - A. Urobilinogen
 - B. Nitrite
 - C. Porphobilinogen
 - D. Leukocyte esterase
- **42.** The reagent strip test for nitrite used the:
 - A. Greiss reaction
 - B. Hoesch reaction
 - C. Peroxidase reaction
 - D. Pseudoperoxidase reaction
- **43.** All of the following can cause a negative nitrite reading *except*:
 - A. Gram-positive bacteria
 - B. Gram-negative bacteria
 - C. Random urine specimens
 - D. Heavy bacterial infections
- **44.** A positive nitrite test and a negative leukocyte esterase test is an indication of a:
 - A. Dilute random specimen
 - B. Specimen with lyzed leukocytes
 - C. Vaginal yeast infection
 - D. Specimen older than 2 hours
- **45.** All of the following can be detected by the leukocyte esterase reaction *except*:
 - A. Neutrophils
 - B. Eosinophils
 - C. Lymphocytes
 - D. Basophils
- **46.** Screening tests for urinary infection combine the leukocyte esterase test with the test for:
 - A. pH
 - B. Nitrite
 - C. Protein
 - D. Blood
- **47**. The principle of the leukocyte esterase reagent strip test uses a:
 - A. Peroxidase reaction
 - B. Double indicator reaction
 - C. Diazo reaction
 - D. Dye-binding technique

- **48.** The principle of the reagent strip test for specific gravity uses the dissociation constant of a/an:
 - A. Diazonium salt
 - B. Indicator dye
 - C. Polyelectrolyte
 - D. Enzyme substrate
- **49.** A specific gravity of 1.030 would produce the reagent strip color:
 - A. Blue
 - B. Green
 - C. Yellow
 - D. Red
- **50**. Reagent strip—specific gravity readings are affected by:
 - A. Glucose
 - B. Radiographic dye
 - C. Alkaline urine
 - D. All of the above

Case Studies and Clinical Situations

1. A patient taken to the emergency room following an episode of syncope has a fasting blood glucose level of 450 mg/dL. Results of the routine urinalysis are as follows:

COLOR: Pale yellow
CLARITY: Clear
SP. GRAVITY: 1.020
BILIRUBIN: Negative
BLOOD: Negative
BILIRUBIN: Negative
UROBILINOGEN: Negative
PROTEIN: 1+
GLUCOSE: 250 mg/dL

KETONES: Negative
BLOOD: Negative
LEUKOCYTES: Negative

- a. Explain the correlation between the patient's blood and urine glucose results.
- b. What is the most probable metabolic disorder associated with this patient?
- c. Considering the patient's condition, what is the significance of the patient's protein result?
- d. What could have been done to delay the onset of proteinuria in this patient?
- e. If the patient in this study had a normal blood glucose level, to what would the urinary glucose be attributed?
- **2.** Results of a urinalysis performed on a patient scheduled for gallbladder surgery are as follows:

COLOR: Amber KETONES: Negative
CLARITY: Hazy BLOOD: Negative
SP. GRAVITY: 1.022 BILIRUBIN: Moderate
ph: 6.0 UROBILINOGEN: Normal
PROTEIN: Negative NITRITE: Negative
GLUCOSE: Negative LEUKOCYTES: Negative

- a. What would be observed if this specimen were shaken?
- b. What confirmatory test could be performed on this specimen?
- c. Explain the correlation between the patient's scheduled surgery and the normal urobilinogen.
- d. If blood were drawn from this patient, how might the appearance of the serum be described?
- e. What special handling is needed for serum and urine specimens from this patient?
- **3.** Results of a urinalysis on a very anemic and jaundiced patient are as follows:

COLOR: Red

CLARITY: Clear

SP. GRAVITY: 1.020

PH: 6.0

PROTEIN: Negative

GLUCOSE: Negative

a. Would these results be indicative of hematuria or hemoglobinuria?

KETONES: Negative

BLOOD: Large

BILIRUBIN: Negative

UROBILINOGEN: 12 EU

NITRITE: Negative

LEUKOCYTES: Negative

a. Would these results be indicative of hematuria or hemoglobinuria?

- b. Correlate the patient's condition with the urobilinogen result.
- c. Why is the urine bilirubin result negative in this jaundiced patient?
- d. If interference by porphyrins was suspected in this specimen, how could this be resolved? State two methods.
- 4. A female patient arrives at the outpatient clinic with symptoms of lower back pain and urinary frequency with a burning sensation. She is a firm believer in the curative powers of vitamins. She has tripled her usual dosage of vitamins in an effort to alleviate her symptoms; however, the symptoms have persisted. She is given a sterile container and asked to collect a midstream clean-catch urine specimen. Results of this routine urinalysis are as follows:

COLOR: Dark yellow
CLARITY: Hazy
SP. GRAVITY: 1.012
BILIRUBIN: Negative
PH: 7.0
UROBILINOGEN: Normal
PROTEIN: Trace
GLUCOSE: Negative
LEUKOCYTES: 1+

Microscopic

8 to 12 rbc/hpf Heavy bacteria

40 to 50 wBC/HPF Moderate squamous epithelial cells

- a. What discrepancies between the chemical and microscopic test results are present? State and explain a possible reason for each discrepancy.
- b. What additional chemical tests could be affected by the patient's vitamin dosage? Explain the principle of the interference.

Continued

- c. Discuss the urine color and specific gravity results with regard to correlation and give a possible cause for any discrepancy.
- d. State three additional reasons not previously given for a negative nitrite test in the presence of increased bacteria.
- **5.** Results of a urinalysis collected following practice from a 20-year-old college athlete are as follows:

COLOR: Dark yellow
CLARITY: Hazy
SP. GRAVITY: 1.029
BILIRUBIN: Negative
PH: 6.5
UROBILINOGEN: 1 EU
PROTEIN: 2+
NITRITE: Negative
GLUCOSE: Negative
LEUKOCYTES: Negative
The physician requests that the athlete collect
another specimen in the morning prior to classes and practice.

- a. What is the purpose of the second sample?
- b. What changes would you expect in the second sample?
- c. Is the proteinuria present in the first sample of prerenal, renal, or postrenal origin?
- 6. A construction worker is pinned under collapsed scaffolding for several hours prior to being taken to the emergency room. His abdomen and upper legs are severely bruised, but no fractures are detected. A specimen for urinalysis obtained by catheterization has the following results:

COLOR: Red-brown
CLARITY: Clear
SP. GRAVITY: 1.017
BILIRUBIN: Negative
PH: 6.5
UROBILINOGEN: 0.4 EU
PROTEIN: Trace
GLUCOSE: Negative
A. Would hematuria be suspected in this specimen?

- Why or why not?
 b. What is the most probable cause of the positive
- c. What is the source of the substance causing the positive blood reaction and the name of the condition?

blood reaction?

- d. Would this patient be monitored for changes in renal function? Why or why not?
- 7. Considering the correct procedures for care, technique, and quality control for reagent strips, state a possible cause for each of the following scenarios.
 - a. The urinalysis supervisor notices that an unusually large number of reagent strips are becoming discolored before the expiration date has been reached.
 - b. A physician's office is consistently reporting positive nitrite test results with negative LE test results.
 - c. A student's results for reagent strip blood and LE are consistently lower than those of the laboratory staff.











Microscopic Examination of Urine

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

- 1 List the physical and chemical parameters included in macroscopic urine screening, and state their significance.
- 2 Discuss the advantages of commercial systems over the glass-slide method for sediment examination
- 3 Describe the recommended methods for standardizing specimen preparation and volume, centrifugation, sediment preparation, volume and examination, and reporting of results.
- 4 State the purpose of Sternheimer-Malbin, acetic acid, toluidine blue, Sudan III, Gram, Hansel, and Prussian blue stains in the examination of the urine sediment.
- 5 Identify specimens that should be referred for cytodiagnostic testing.
- 6 Describe the basic principles of bright-field, phase-contrast, polarizing, dark-field, fluorescence, and interference-contrast microscopy, and their relationship to sediment examination.
- 7 Differentiate between normal and abnormal sediment constituents.
- 8 Discuss the significance of red blood cells (RBCs) in the urinary sediment.

- **9** Discuss the significance of white blood cells (WBCs) in the urinary sediment.
- 10 Name, describe, and give the origin and significance of the three types of epithelial cells found in the urinary sediment.
- 11 Discuss the significance of oval fat bodies.
- 12 Describe the process of cast formation.
- 13 Describe and discuss the significance of hyaline, RBC, WBC, bacterial, epithelial cell, granular, waxy, fatty, and broad casts.
- **14** List and identify the normal crystals found in acidic urine.
- 15 List and identify the normal crystals found in alkaline urine.
- 16 Describe and state the significance of cystine, cholesterol, leucine, tyrosine, bilirubin, sulfonamide, radiographic dye, and ampicillin crystals.
- **17** Differentiate between actual sediment constituents and artifacts.
- 18 Correlate physical and chemical urinalysis results with microscopic observations and recognize discrepancies.

KEY TERMS

bright-field microscopy casts chemical sieving cylindruria

dark-field microscopy fluorescence microscopy interference-contrast microscopy phase-contrast microscopy
polarizing microscopy
resolution
Tamm-Horsfall protein

82 CHAPTER 6 • Microscopic Examination of Urine

The third part of routine urinalysis is the microscopic examination of the urinary sediment. Its purpose is to detect and to identify insoluble materials present in the urine. The blood, kidney, lower genitourinary tract, and external contamination all contribute formed elements to the urine. These include RBCs, WBCs, epithelial cells, casts, bacteria, yeast, parasites, mucus, spermatozoa, crystals, and artifacts. Because some of these components are of no clinical significance and others are considered normal unless they are present in increased amounts, examination of the urinary sediment must include both identification and quantitation of the elements present. Microscopic examination of the urine sediment is the least standardized and most time-consuming part of the routine urinalysis. Protocols have been developed to increase the standardization and cost-effectiveness of microscopic urinalysis and are discussed in this chapter.

Macroscopic Screening

To enhance the cost-effectiveness of urinalysis, many laboratories have developed protocols, whereby microscopic examination of the urine sediment is performed only on specimens meeting specified criteria. Abnormalities in the physical and chemical portions of the urinalysis play a primary role in the decision to perform a microscopic analysis, thus the use of the term macroscopic screening, also referred to as chemical sieving. Parameters considered significant vary among laboratories but usually include color, clarity, blood, protein, nitrite, leukocyte esterase, and possibly glucose. Laboratory-designated criteria can also be programed into automated instruments. Table 6-1 illustrates the significance of these parameters. Percentages of abnormal specimens that would go undetected using these parameters differ significantly among studies.^{1,2} The patient population must also be considered when developing protocols for macroscopic screening. Populations that have come under consideration include pregnant women, as well as

The second secon	Aacroscopic Screening Correlations
Screening Test	Significance
Color	Blood
Clarity	Hematuria versus hemoglobin- uria/myoglobinuria Confirm pathologic or non- pathologic cause of turbidity
Blood	RBCs/RBC casts
Protein	Casts/cells
Nitrite	Bacteria/WBCs
Leukocyte esterase	WBCs/WBC casts/bacteria
Glucose	Yeast

pediatric, geriatric, diabetic, immunocompromised, and renal patients. The Clinical and Laboratory Standards Institute (CLSI) recommends that microscopic examination be performed when requested by a physician, when a laboratory-specified patient population is being tested, or when any abnormal physical or chemical result is obtained.³

Preparation and Examination of the Urine Sediment

Microscopic analysis is subject to several procedural variations, including the methods by which the sediment is prepared, the volume of sediment actually examined, the methods and equipment used to obtain visualization, and the manner in which the results are reported. The first procedure to standardize the quantitation of formed elements in the urine microscopic analysis was developed by Addis in 1926. The Addis count, as it is called, used a hemocytometer to count the number of RBCs, WBCs, casts, and epithelial cells present in a 12-hour specimen. Normal values have a wide range and are approximately 0 to 500,000 RBCs, 0 to 1,800,000 WBCs and epithelial cells, and 0 to 5000 hyaline casts.4 The Addis count, which was used primarily to monitor the course of diagnosed cases of renal disease, has been replaced by various standardized commercial systems for the preparation, examination, and quantitation of formed elements in nontimed specimens.

Commercial Systems

The conventional method of placing a drop of centrifuged urine on a glass slide, adding a cover slip, and examining microscopically has been substantially improved through the use of commercial slide systems.5 The CLSI recommends their use together with standardization of all phases of the methodology, including the conventional method, as discussed in the following sections. Systems currently available include KOVA (Hycor Biomedical, Inc., Garden Grove, Calif.), Urisystem (ThermoFisher Scientific, Waltham, Mass.), Count-10 (V-Tech, Inc., Pomona, Calif.), Quick-Prep Urinalysis System (Globe Scientific, Paramus, N.J.), CenSlide 2000 Urinalysis System (International Remote Imaging Systems, Norwood, Mass.), and R/S Workstations 1000, 2000, 2003 (DioSys, Waterbury, Calif.). The systems provide a variety of options including capped, calibrated centrifuge tubes; decanting pipettes to control sediment volume; and slides that control the amount of sediment examined, produce a consistent monolayer of sediment for examination, and provide calibrated grids for more consistent quantitation.

The Cen-Slide and R/S Workstations do not require manual loading of the centrifuged specimen onto a slide and are considered closed systems that minimize exposure to the specimen. Cen-Slide provides a specially designed tube that permits direct reading of the urine sediment. The R/S Workstations consist of a glass flow cell into which urine sediment is pumped, microscopically examined, and then flushed from the system.

Specimen Preparation

Specimens should be examined while fresh or adequately preserved. Formed elements—primarily RBCs, WBCs, and hyaline casts—disintegrate rapidly, particularly in dilute alkaline urine. Refrigeration may cause precipitation of amorphous urates and phosphates and other nonpathologic crystals that can obscure other elements in the urine sediment. Warming the specimen to 37°C prior to centrifuging may dissolve some of these crystals.

The midstream clean-catch specimen minimizes external contamination of the sediment. As with the physical and chemical analyses, dilute random specimens may cause falsenegative readings.

Care must be taken to thoroughly mix the specimen prior to decanting a portion into a centrifuge tube.

Specimen Volume

A standard amount of urine, usually between 10 and 15 mL, is centrifuged in a conical tube. This provides an adequate volume from which to obtain a representative sample of the elements present in the specimen. A 12-mL volume is frequently used because multiparameter reagent strips are easily immersed in this volume, and capped centrifuge tubes are often calibrated to this volume.

If obtaining a 12-mL specimen is not possible, as with pediatric patients, the volume of the specimen used should be noted on the report form. This allows the physician to correct the results, if indicated. Some laboratories choose to make this correction prior to reporting. For example, if 6 mL of urine is centrifuged, the results are multiplied by 2.

Centrifugation

The speed of the centrifuge and the length of time the specimen is centrifuged should be consistent. Centrifugation for 5 minutes at a relative centrifugal force (RCF) of 400 produces an optimum amount of sediment with the least chance of damaging the elements. To correct for differences in the diameter of centrifuge heads, RCF rather than revolutions per minute (RPM) is used. The RPM value shown on the centrifuge tachometer can be converted to RCF using nomograms available in many laboratory manuals or by using the formula:

 $RCF = 1.118 \times 10^{-5} \times radius in centimeters \times RPM^2$

Centrifugation calibration should be routinely performed. Use of the braking mechanism to slow the centrifuge causes disruption of the sediment prior to decantation and should not be used.

To prevent biohazardous aerosols, all specimens must be centrifuged in capped tubes.

Sediment Preparation

A uniform amount of urine and sediment should remain in the tube after decantation. Volumes of 0.5 and 1.0 mL are fre-

quently used. The volume of urine centrifuged divided by the sediment volume equals the concentration factor, which in the preceding examples is 24 and 12, respectively. The sediment concentration factor relates to the probability of detecting elements present in low quantities and is used when quantitating the number of elements present per milliliter.

To maintain a uniform sediment concentration factor, urine should be aspirated off rather than poured off, unless otherwise specified by the commercial system in use. Some systems provide pipettes for this purpose. The pipettes are also used for sediment resuspension and transfer of specimens to the slide.

The sediment must be thoroughly resuspended by gentle agitation. This can be performed using a commercial-system pipette or by repeatedly tapping the tip of the tube with the finger. Vigorous agitation should be avoided, as it may disrupt some cellular elements. Thorough resuspension is essential to provide equal distribution of elements in the microscopic examination fields.

Volume of Sediment Examined

The volume of sediment placed on the microscope slide should be consistent for each specimen. When using the conventional glass-slide method, the recommended volume is 20 μ L (0.02 mL) covered by a 22 \times 22 mm glass cover slip. Allowing the specimen to flow outside of the cover slip may result in the loss of heavier elements such as casts.

Commercial systems control the volume of sediment examined by providing slides with chambers capable of containing a specified volume. Care must be taken to ensure the chambers are completely filled. Product literature supplies the chamber volume, size of the viewing area, and approximate number of low-power and high-power viewing areas, based on the area of the field of view using a standard microscope. This information, together with the sediment concentration factor, is necessary to quantitate cellular elements per milliliter of urine.

Examination of the Sediment

The manner by which the microscopic examination is performed should be consistent and should include observation of a minimum of 10 fields under both low $(10\times)$ and high $(40\times)$ power. The slide is first examined under low power to detect casts and to ascertain the general composition of the sediment. When elements such as casts that require identification are encountered, the setting is changed to high power.

If the conventional glass-slide method is being used, casts have a tendency to locate near the edges of the cover slip; therefore, low-power scanning of the cover-slip perimeter is recommended. This does not occur when using standardized commercial systems.

When the sediment is examined unstained, many sediment constituents have a refractive index similar to urine. Therefore, it is essential that sediments be examined under reduced light when using **bright-field microscopy**.

Initial focusing can be difficult with a fluid specimen, and care must be taken to ensure that the examination is

84

being performed in the correct plane. Often an epithelial cell will be present to provide a point of reference. Focusing on artifacts should be avoided, because they are often larger than the regular sediment elements and cause the microscopist to examine objects in the wrong plane. Continuous focusing with the fine adjustment aids in obtaining a complete representation of the sediment constituents.

Reporting the Microscopic Examination

The terminology and methods of reporting may differ slightly among laboratories but must be consistent within a particular laboratory system. Routinely, casts are reported as the average number per low-power field (**lpf**) following examination of 10 fields, and RBCs and WBCs, as the average number per 10 high-power fields (**hpfs**). Epithelial cells, crystals, and other elements are frequently reported in semiquantitative terms such as, rare, few, moderate, and many, or as 1+, 2+, 3+, and 4+, following laboratory format as to lpf or hpf use. Laboratories must also determine their particular reference values based on the sediment concentration factor in use. For example, Urisystem, with a concentration factor of 30, states a reference value for WBCs of zero to eight per hpf, as opposed to the conventional value of zero to five per hpf used with a concentration factor of 12.

Conversion of the average number of elements per lpf or hpf to the number per milliliter provide standardization among the various techniques in use. Steps include the following:

Example

1. Calculation of the area of an lpf or hpf for the microscope in use using the manufacturer-supplied field of view diameter and the formula $\pi r^2 = \text{area}$. Diameter of hpf = 0.35 mm

$$3.14 \times 0.175^2 = 0.096 \text{ mm}^2$$

2. Calculation of the maximum number of lpfs or hpfs in the viewing area.

Area under a 22 mm
$$\times$$
 22 mm cover slip = 484 mm²

$$\frac{484}{.096} = 5040 \text{ hpfs}$$

3. Calculation of the number of hpss per milliliter of urine tested using the concentration factor and the volume of sediment examined.

$$\frac{5040}{0.02 \text{ mL} \times 12} = \frac{5040}{.24} = 21,000 \text{ hpf/mL of urine}$$

4. Calculation of the number of formed elements per milliliter of urine by multiplying the number of hpfs per milliliter by the average number of formed elements per field.

 $4 \text{ WBC/hpf} \times 21,000 = 84,000 \text{ WBC/mL}$

Provided the same microscope and volume of sediment examined are used, the number of lpfs and hpfs per milliliter of urine remains the same, thereby simplifying the calculation.

Laboratories should evaluate the advantages and disadvantages of adding an additional calculation step to the microscopic examination. The CLSI states that all decisions with regard to reporting of the microscopic should be based on the needs of the individual laboratory. Procedures should be completely documented and followed by all personnel.

Correlation of Results

Microscopic results should be correlated with the physical and chemical findings to ensure the accuracy of the report. Specimens in which the results do not correlate must be rechecked for both technical and clerical errors. Table 6–2 shows some of the more common correlations in the urinalysis; however, the amount of formed elements or chemicals must also be considered, as must the possibility of interference with chemical tests and the age of the specimen.

Sediment ExaminationTechniques

Many factors can influence the appearance of the urinary sediment, including cells and casts in various stages of development and degeneration, distortion of cells and crystals by the chemical content of the specimen, the presence of inclusions in cells and casts, and contamination by artifacts. Therefore, identification can sometimes be difficult even for experienced laboratory personnel. Identification can be enhanced through the use of sediment stains (Table 6–3) and different types of microscopy.

Table 6-2 Routine Urinalysis Correlations			
Microscopic Elements	Physical	Chemical	Exceptions
RBCs	Turbidity Red color	+ Blood	Number Hemolysis
WBCs	Turbidity	+ Protein + Nitrite + Leukocytes	Number Lysis
Epithelial cells	Turbidity		Number
Casts		+ Protein	Number
Bacteria	Turbidity	pH + Nitrite + Leukocytes	Number and type
Crystals	Turbidity Color	рН	Number and type

Table 6-3 Sediment Stain Characteristics			
Stain	Action	Function	
Sternheimer-Malbin	Delineates structure and contrasting colors of the nucleus and cytoplasm	Identifies WBCs, epithelial cells, and casts	
Toluidine blue	Enhances nuclear detail	Differentiates WBCs and renal tubular epithelial (RTE) cells	
2% acetic acid	Lyses RBCs and enhances nuclei of WBCs	Distinguishes RBCs from WBCs, yeast, oil droplets, and crystals	
Lipid Stains: Oil Red O and Sudan III	Stains triglycerides and neutral fats orange-red	Identifies free fat droplets and lipid-containing cells and casts	
Gram stain	Differentiates gram-positive and gram- negative bacteria	Identifies bacterial casts	
Hansel stain	Methylene blue and eosin Y stains eosinophilic granules	Identifies urinary eosinophils	
Prussian blue stain	Stains structures containing iron	Identifies yellow-brown granules of hemo- siderin in cells and casts	

Sediment Stains

Staining increases the overall visibility of sediment elements being examined using bright-field microscopy by changing their refractive index. As mentioned, elements such as hyaline casts have a refractive index very similar to that of urine. Staining also imparts identifying characteristics to cellular structures, such as the nuclei, cytoplasm, and inclusions.

The most frequently used stain in urinalysis is the Sternheimer-Malbin stain, which consists of crystal violet and safranin O.⁶ The stain is available commercially under a variety of names, including Sedi-Stain (Becton-Dickinson, Parsippany, N.J.) and KOVA stain (Hycor Biomedical, Inc, Garden Grove, Calif.). Commercial brands contain stabilizing chemicals to prevent the precipitation that occurred with the original stain. The dye is absorbed well by WBCs, epithelial cells, and casts, providing clearer delineation of structure and contrasting colors of the nucleus and cytoplasm. Table 6–4 provides an example of the staining reactions as shown in the product literature.

A 0.5% solution of toluidine blue, a metachromatic stain, provides enhancement of nuclear detail. It can be useful in the differentiation between WBCs and renal tubular epithelial cells and is also used in the examination of cells from other body fluids.

Nuclear detail is also enhanced by the addition of 2% acetic acid to the sediment. This method cannot be used for initial sediment analysis because RBCs are lysed by the acetic acid.

Lipid Stains

The passage of lipids (triglycerides, neutral fats, and cholesterol) across the glomerular membrane results in the appear-

ance of free fat droplets and lipid-containing cells and casts in the urinary sediment. The lipid stains, Oil Red O and Sudan III, and polarizing microscopy can be used to confirm the presence of these elements. Triglycerides and neutral fats stain orange-red in the presence of stain, whereas cholesterol does not stain but is capable of polarization. The three lipids are usually present concurrently in the sediment, thereby permitting use of either staining or polarization for their confirmation.

Gram Stain

The Gram stain is used primarily in the microbiology section for the differentiation between gram-positive (blue) and gram-negative (red) bacteria. Its role in routine urinalysis is limited to the identification of bacterial casts, which can easily be confused with granular casts. To perform Gram staining, a dried, heat-fixed preparation of the urine sediment must be used.

Hansel Stain

Polynuclear WBCs seen in the urinary sediment are almost always neutrophils associated with microbial infection. However, in cases of a drug-induced allergic reaction producing inflammation of the renal interstitium, eosinophils are present in the sediment. The preferred stain for urinary eosinophils is Hansel stain, consisting of methylene blue and eosin Y (Lide Labs, Inc, Florissant, Mo.); however, Wright's stain can also be used. Staining is performed on a dried smear of the centrifuged specimen or a cytocentrifuged preparation of the sediment.

Prussian Blue Stain

As discussed in Chapter 5, following episodes of hemoglobinuria, yellow-brown granules may be seen in renal tubular

Elements in Urinary Sediment	Usual Distinguishing Color of Stained Elements		Comments
RBCs	Neutral—pink to purple Acid—pink (unstained) Alkaline—purple <i>Nuclei</i>	Cytoplasm	
WBCs (dark-staining cells)	Purple	Purple granules	
Glitter cells (Sternheimer- Malbin positive cells)	Colorless or light blue	Pale blue or gray	Some glitter cells exhibit brownian movement
Renal tubular epithelial cells	Dark shade of blue-purple	Light shade of blue-purple	
Bladder tubular epithelial cells	Blue-purple	Light purple	
Squamous epithelial cells	Dark shade of orange-purple Inclusions and Matrix	Light purple or blue	
Hyaline casts	Pale pink or pale purple		Very uniform color; slightly darker than mucous threads
Coarse granular inclusion casts	Dark purple granules in purple matrix		
Finely granular inclusion casts	Fine dark purple granules in pale pink or pale purple matrix		
Waxy casts	Pale pink or pale purple		Darker than hyaline casts, bu of a pale even color; distinct broken ends
Fat inclusion casts	Fat globules unstained in a pink matrix		Rare; presence is confirmed i examination under polarized light indicates double refraction
Red cell inclusion casts	Pink to orange-red		Intact cells can be seen in matrix
Blood (hemoglobin) casts	Orange-red		No intact cells
Bacteria	Motile: do not stain Nonmotile: stain purple		Motile organisms are not impaired
Trichomonas vaginalis	Light blue-green		Motility is unimpaired in fresh specimens when recom mended volumes of stain are used; immobile organisms also identifiable
Mucus	Pale pink or pale blue		
Background	Pale pink or pale purple		

epithelial cells and casts or free-floating in the urine sediment. To confirm that these granules are hemosiderin, the Prussian blue stain for iron is used and stains the hemosiderin granules a blue color.

Cytodiagnostic Urine Testing

Although it is not a part of the routine examination of the urine sediment, the preparation of permanent slides using cytocentrifugation followed by staining with Papanicolaou stain provides an additional method for detecting and monitoring renal disease. Cytodiagnostic urine testing is frequently performed independently of routine urinalysis for detection of malignancies of the lower urinary tract. A voided first morning specimen is recommended for testing, which is performed by the cytology laboratory. Cytodiagnostic urine testing also provides more definitive information about renal tubular changes associated with transplant rejection; viral, fungal, and parasitic infections; cellular inclusions; pathologic casts; and inflammatory conditions. The urinalysis laboratory should refer specimens with unusual cellular findings to the cytology laboratory for further examination.

Microscopy

Microscopic examination of urine is best performed when the laboratorian is knowledgeable about the types of microscopes available, their primary characteristics, and the proper use and maintenance of these microscopes.

Bright-field microscopy is the most common type of microscopy performed in the urinalysis laboratory. Other types of microscopy that are useful for examining the urine sediment are phase contrast, polarizing, dark field, fluorescence, and interference contrast (Table 6–5). The type of microscopy used depends on the specimen type, the refractive index of the object, and the ability to image unstained living cells. All microscopes are designed to magnify small objects to such a degree that the details of their structure can be analyzed. Basically, they do this by employing a variety of lenses and light sources as described in the following section.

The Microscope

Essentially all types of microscopes contain a lens system, illumination system, and a body consisting of a base, body tube, and nosepiece (Fig. 6-1). Primary components of the lens system are the oculars, the objectives, and the coarse- and fine-adjustment knobs. The illumination system contains the light source, condenser, and field and iris diaphragms. Objects to be examined are placed on a platform, referred to as the mechanical stage. The compound bright-field microscope is used primarily in the urinalysis laboratory and consists of a two-lens system combined with a light source. The first lens system is located in the objective and is adjusted to be near the specimen. The second lens system, the ocular lens, is located in the eyepiece. The path of light passes through the specimen up to the eyepiece.

Table 6–5	Urinalysis Microscopic Techniques
Technique	Function
Bright-field microscopy	Used for routine urinalysis
Phase-contrast microscopy	Enhances visualization of elements with low refractive indices, such as hyaline casts, mixed cellular casts, mucous threads, and <i>Trichomonas</i>
Polarizing microscopy	Aids in identification of cholesterol in oval fat bodies, fatty casts, and crystals
Dark-field microscopy	Aids in identification of <i>Treponema pallidum</i>
Fluorescence microscopy	Allows visualization of naturally fluorescent microorganisms or those stained by a fluorescent dye
Interference- contrast	Produces a three-dimensional microscopy-image and layer-by-layer imaging of a specimen

The oculars or eyepieces of the microscope are located at the top of the body tube. Clinical laboratory microscopes are binocular, allowing the examination to be performed using both eyes to provide more complete visualization. For optimal viewing conditions, the oculars can be adjusted horizontally to adapt to differences in interpupillary distance between operators. A diopter adjustment knob on the oculars can be rotated to compensate for variations in vision between the operators' eyes. The oculars are designed to further magnify the object that has been enhanced by the objectives for viewing. Laboratory microscopes normally contain oculars capable of increasing the magnification 10 times (10×). The field of view is determined by the eyepiece and is the diameter of the

PROCEDURE



Care of the Microscope

- 1. Carry microscope with two hands, supporting the base with one hand.
- 2. Always hold the microscope in a vertical position.
- 3. Only clean optical surfaces with a good quality lens tissue and commercial lens cleaner.
- 4. Do not use the $10 \times$ and $40 \times$ objectives with oil.
- 5. Clean the oil immersion lens after use.
- **6.** Always remove slides with the low-power objective raised.
- Store the microscope with the low-power objective in position and the stage centered.

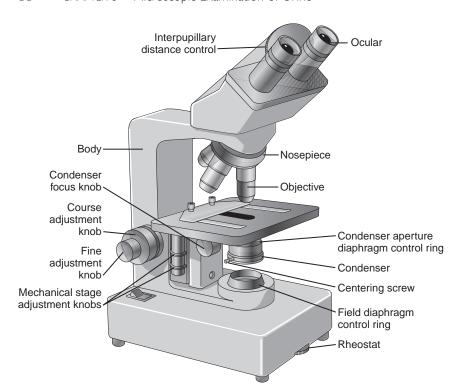


Figure 6–1 Parts of the binocular microscope.

circle of view when looking through the oculars. The field of view varies with the field number engraved on the eyepiece and the magnification of the objective. The higher the magnification, the smaller the field of view. In urinalysis microscopy, sediment constituents are reported as the number per microscopic field (number per hpf or lpf).

Objectives are contained in the revolving nosepiece located above the mechanical stage. Objectives are adjusted to be near the specimen and perform the initial magnification of the object on the mechanical stage. The image then passes to the oculars for further resolution (ability to visualize fine details). Resolution is the ability of the lens to distinguish two small objects that are a specific distance apart. Resolving power is best when the distance between the two objects is small. It is dependent on the wavelength of light and the numerical aperture of the lens. The shorter the wavelength of light, the greater the resolving power of the microscope. Routinely used objectives in the clinical laboratory have magnifications of 10× (low power, dry), 40× (high power, dry), and 100× (oil immersion). The objectives used for examination of urine sediment are 10× and 40×. The final magnification of an object is the product of the objective magnification times the ocular magnification. Using a 10× ocular and a 10× objective provides a total magnification of 100× and in urinalysis is the lpf observation. The 10× ocular and the 40× objective provide a magnification of 400× for hpf observations.

Objectives are inscribed with information that describes their characteristics and includes the type of objective (plan used for bright field, ph for phase contrast), magnification, numerical aperture, microscope tube length, and cover-slip thickness to be used. The numerical aperture number repre-

sents the refractive index of the material between the slide and the outer lens (air or oil) and the angle of the light passing through it. The higher the numerical aperture, the better the light-gathering capability of the lens, yielding greater resolving power. The length of the objectives attached to the nosepiece varies with magnification (length increases from $10\times$ to $100\times$ magnification), thereby changing the distance between the lens and the slide when they are rotated. The higher the numerical aperture, the closer the lens is to the object. Most microscopes are designed to be parfocal, indicating that they require only minimum adjustment when switching among objectives.

The distance between the slide and the objective is controlled by the coarse and fine focusing knobs located on the body tube. Initial focusing is performed using the coarse knob that moves the mechanical stage noticeably up and down until the object comes into view. This is followed by adjustment using the fine focusing knob to sharpen the image. When using a parfocal microscope, only the fine knob should be used for adjustment when changing magnifications.

Illumination for the modern microscope is provided by a light source located in the base of the microscope. The light source is equipped with a rheostat to regulate the intensity of the light. Filters may also be placed on the light source to vary the illumination and wavelengths of the emitted light. A field diaphragm contained in the light source controls the diameter of the light beam reaching the slide and is adjusted for optimal illumination. A condenser located below the stage then focuses the light on the specimen and controls the light for uniform illumination. The normal position of the condenser is almost completely up with the front lens of the con-

denser near the slide but not touching it. The condenser adjustment (focus) knob moves the condenser up and down to focus light on the object. An aperture diaphragm in the condenser controls the amount of light and the angle of light rays that pass to the specimen and lens, which affects resolution, contrast, and depth of the field of image. By adjusting the aperture diaphragm to 75% of the numerical aperture of the objective, maximum resolution is achieved. The aperture diaphragm should not be used to reduce light intensity because it decreases resolution. The microscope lamp rheostat is used for this adjustment.

Köhler Illumination

Two adjustments to the condenser—centering and Köhler illumination—provide optimal viewing of the illuminated field. They should be performed whenever an objective is changed. To center the condenser and obtain Köhler illumination, the following steps should be taken:

- Place a slide on the stage and focus the object using the low-power objective with the condenser raised.
- Close the field diaphragm.
- Lower the condenser until the edges of the field diaphragm are sharply focused.
- Center the image of the field diaphragm with the condenser centering screws as shown in Figure 6-2, part A.
- Open the field diaphragm until its image is at the edge of the field.
- Remove an eyepiece and look down through the eyepiece tube.
- Adjust the aperture diaphragm until approximately 75% of the field is visible (see Fig. 6-2, part B).
- Replace the eyepiece.

Additional focusing of the object should be performed using the adjustment knobs and the rheostat on the light source.

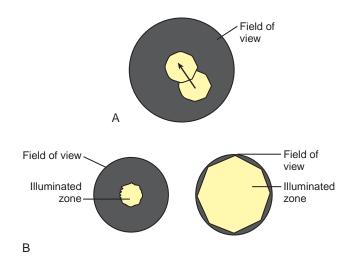


Figure 6–2 Centering the condenser and Köhler illumination.

Routine preventive maintenance procedures on the microscope ensure good optical performance. The microscope should always be covered when not in use to protect it from dust. If any optical surface becomes coated with dust, it should be carefully removed with a camel-hair brush. Optical surfaces should be cleaned with lens paper. Clean any contaminated lens immediately with a commercial lens cleaner. An oil immersion lens must be wiped free of oil and cleaned after each use. Fingerprints and oil smears impair the sharpness of an image. An annual professional cleaning for the microscope is recommended. Light sources are replaced as necessary.

Types of Microscopy

Bright-Field Microscopy

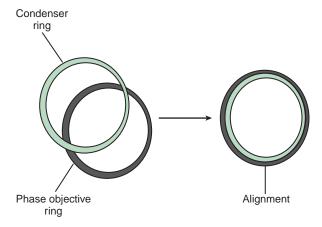
Bright-field microscopy, in which objects appear dark against a light background, is most frequently used in the clinical laboratory. This technique employs the basic microscope previously described with a light source emitting light in the visible wavelength range.

Use of bright-field microscopy for the examination of urine sediment can present problems when the amount of light reaching the specimen is not properly controlled. Sediment constituents with a low refractive index will be overlooked when subjected to light of high intensity. Therefore, sediments must be examined using decreased light controlled by adjusting the rheostat on the light source, not by lowering the condenser. Staining of the sediment also increases the visualization of these elements when using bright-field microscopy.

Phase-Contrast Microscopy

As light rays pass through an object, they are slowed in comparison to the rays passing through air (media), thereby decreasing the intensity of the light and producing contrast. This is called phase difference and is affected by the thickness of the object, refractive index, and other light-absorbance properties. The best contrast is obtained when the light that does not pass through the specimen is shifted one quarter of a wavelength and compared with the phase difference of the specimen. *Phase-contrast microscopy* provides this contrast.

Phase-contrast microscopy is accomplished by adaptation of a bright-field microscope with a phase-contrast objective lens and a matching condenser. Two phase rings that appear as "targets" are placed in the condenser and the objective. One phase ring is placed in the condenser or below it, permitting light to only pass through the central clear circular area. A second phase-shifting ring with a central circular area that retards the light by one quarter wavelength is placed in the objective. Phase rings must match, so it is important to check that the objective and condenser mode are the same. The diameter of the rings varies with the magnification. The image has the best contrast when the background is darkest. Phase-contrast rings must be adjusted to have maximum contrast. The two rings are adjusted to make them concentric. Adjustment steps are as follows:⁷



Centering phase microscope rings

Figure 6-3 Phase-contrast ring adjustment.

- Focus the microscope in bright-field with a specimen slide.
- Select a low-power phase condenser ring.
- Select the corresponding ring objective.
- Remove an ocular, insert the adjustment telescope, and look through the telescope.
- Observe the dark and light rings (annuli).
- With the adjusting screw on the telescope, center the light annulus (condenser) over the dark annulus (objective) (Fig. 6-3).
- · Replace the ocular.

Light passes to the specimen through the clear circle in the phase ring in the condenser, forming a halo of light around the specimen. The diffracted light then enters the central circle of the phase-shifting ring, and all other light is moved one quarter of a wavelength out of phase. The variations of contrast in the specimen image due to the various refractive indexes in the object are observed as the light rays merge together, enhancing visualization and detail. Phase-contrast microscopy is particularly advantageous for identifying low refractive hyaline casts or mixed cellular casts, mucous threads, and *Trichomonas*. It eliminates the need to fix or stain living cells.

Polarizing Microscopy

The use of polarized light aids in the identification of crystals and lipids. Both substances have the ability to rotate the path of the unidirectional polarized light beam to produce characteristic colors in crystals and Maltese cross formation in lipids. These elements seen under polarized light microscopy are *birefringent*, a property indicating that the element can refract light in two dimensions at 90 degrees to each other.

The halogen quartz lamp in the microscope produces light rays of many different waves. Each wave has a distinct direction and a vibration perpendicular to its direction. Normal or unpolarized light vibrates in equal intensity in all directions. Polarized light vibrates in the same plane or direction. As the light passes through a birefringent substance, it splits

into two beams, one beam rotated 90 degrees to the other. Isotropic substances such as blood cells do not have this refractive property, and the light passes through unchanged. A substance that rotates the plane of polarized light 90 degrees in a clockwise direction is said to have positive birefringence. In contrast, a substance that rotates the plane in a counterclockwise direction has negative birefringence.

Polarized light is obtained by using two polarizing filters. The light emerging from one filter vibrates in one plane, and a second filter placed at a 90-degree angle blocks all incoming light, except that rotated by the birefringent substance. The filters are in opposite directions called a "crossed configuration." Between cross-polarizing filters, birefringent crystals are visible in characteristic patterns (Fig. 6-4).

Bright-field microscopes can be adapted for *polarizing microscopy*. Two polarizing filters must be installed in a crossed configuration. The first filter, the polarizing filter, is placed in the condenser filter holder; the second filter, the analyzer, is placed in the head between the objectives and the ocular. The polarizing filter is rotated to allow light vibrating in one direction only to reach the object. If the object does not have birefringent properties, no light will reach the analyzer filter and the object will appear black. Refracted rays from a birefringent object will reach the analyzer, causing the object to appear white or colored against the black background.

Polarizing microscopy is used in urinalysis to confirm the identification of fat droplets, oval fat bodies, and fatty casts that produce a characteristic Maltese cross pattern. Birefringent uric acid crystals can be distinguished from cystine crystals, monohydrate calcium oxalate crystals from nonpolarizing RBCs, and calcium phosphate crystals differentiated from nonpolarizing bacteria by their polarizing characteristics. As discussed in Chapter 12, polarized microscopy is a valuable tool for the identification of synovial fluid crystals.

Interference-Contrast Microscopy

Interference-contrast microscopy provides a three-dimensional image showing very fine structural detail by splitting the light ray so that the beams pass through different areas of the specimen. The light interference produced by the varied depths of the specimen is compared, and a three-dimensional image is visualized. The advantage of interference-contrast microscopy is that an object appears bright

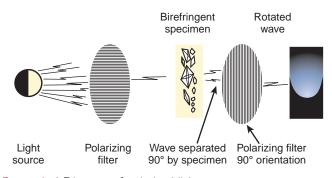


Figure 6–4 Diagram of polarized light.

against a dark background but without the diffraction halo associated with phase-contrast microscopy. More extensive modifications to the bright-field microscope are required to perform this technique. Therefore, it is not routinely used in the urinalysis laboratory.

Two types of interference-contrast microscopy are available: modulation contrast (Hoffman) and differentialinterference contrast (Nomarski). Bright-field microscopes can be adapted for both methods. In the modulation-contrast microscope, a split aperture is placed below the condenser, a polarizer is placed below the split aperture, and an amplitude filter is placed in back of each objective. The modulator has three zones of light transmission: a dark zone that transmits 1% of light, a gray zone that transmits 15% of light, and a clear zone that transmits 100% of light. The polarized light rays pass through a split aperture to the various areas of the specimen and to the modulator where they are converted into the variations of light intensity to produce a three-dimensional image. The differential interference-contrast microscope uses prisms. A polarizing filter to output plane-polarized light is placed between the light source and the condenser. A twolayered Nomarski-modified Wollaston prism that separates individual rays of light into ray pairs is required. The lower Wollaston prism is built into the condenser of the microscope. The upper prism is placed between the objective and the eyepiece and recombines the rays. Above the top Wollaston prism, another polarizing filter is placed that causes wave interference to occur and produce the three-dimensional image (Fig. 6-5).8 These two types of microscopy provide layer-by-layer imaging of a specimen and enhanced detail for specimens with either a low or high refractive index.

Dark-Field Microscopy

Dark-field microscopy is a technique used in the clinical laboratory to enhance visualization of specimens that cannot be seen easily viewed with a bright-field microscope. It is often used for unstained specimens, and, in particular, to identify the spirochete *Treponema pallidum*.

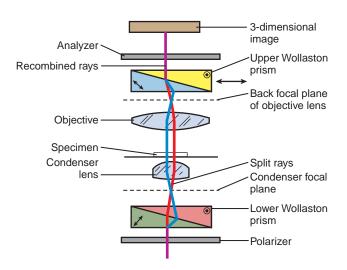


Figure 6–5 Differential interference-contrast (Nomarski) microscopy.

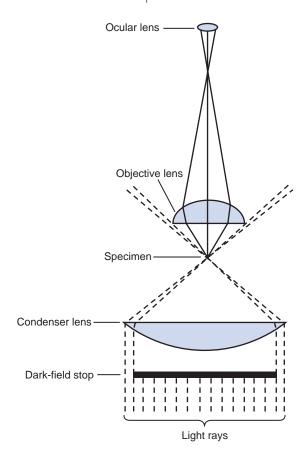


Figure 6-6 Dark-field microscopy.

A bright-field microscope is easily adapted for dark-field microscopy by replacing the condenser with a dark-field condenser that contains an opaque disk. The disk blocks the light from directly entering the objective, and the field of view is black. As the light rays pass through the specimen at oblique angles, the light scatters, diffracts, or reflects off the specimen and is captured by the objective lens. The specimen appears light against the black background or dark-field (Fig. 6-6).

Fluorescence Microscopy

Fluorescence microscopy is a rapidly expanding technique used in the medical field today. It is used to detect bacteria and viruses within cells and tissues through a technique called immunofluorescence. Fluorescence is the property by which some atoms absorb light at a particular wavelength and subsequently emit light of a longer wavelength, termed fluorescence lifetime. The practical application in the laboratory is that it allows the visualization of naturally fluorescent substances or those that have been stained with a fluorochrome or fluorophore (fluorescent dyes) to produce an image. The specimen is illuminated with a light of a specific wavelength. Fluorescent substances absorb the energy and emit a longer wavelength of light that is visualized with the use of special filters, called the excitation filter and the emission filter. The excitation filter selects the excitation wavelength of light from a light source. The emission filter selects a specific wavelength of emitted light from the specimen to become visible. The fil-

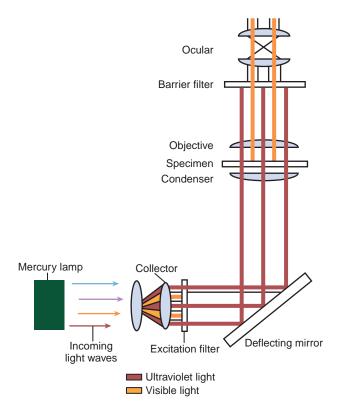


Figure 6-7 Fluorescent microscopy.

ters are chosen to match the excitation and emission wavelengths of the fluorophore used to label the specimen. A dichroic mirror reflects the excitation light to the specimen and transmits the emitted light to the emission filter, which is collected with the objective and imaged by the detector (Fig. 6-7). The fluorescent substance can be observed in the fluorescent microscope as a bright object against a dark background with high contrast when ultraviolet light source is used. Powerful light sources are required and are usually either mercury or xenon arc lamps.⁹

Sediment Constituents

The normal urine sediment may contain a variety of formed elements. Even the appearance of small numbers of the usually pathologically significant RBCs, WBCs, and *casts* can be normal. Likewise, many routine urines contain nothing more than a rare epithelial cell or mucous strand. Students often have difficulty adjusting to this, because in the classroom setting, sediments containing abnormalities and multiple elements are usually stressed. They must learn to trust their observations after looking at the recommended number of fields. Cellular elements are also easily distorted by the widely varying concentrations, pH, and presence of metabolites in urine, making identification more difficult.

Actual normal numerical values are not clearly defined. As discussed previously, sediment preparation methods determine the actual concentration of the sediment and, therefore, the number of elements that may be present in a microscopic field. Commonly listed values include zero to two or three RBCs per hpf, zero to five to eight WBCs per hpf, and zero to

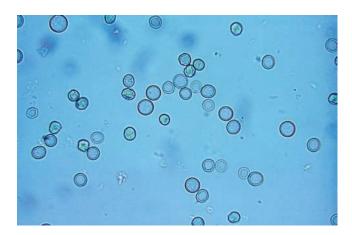


Figure 6–8 Normal RBCs (×400).

two hyaline casts per lpf. Even these figures must be taken in context with other factors, such as recent stress and exercise, menstrual contamination, and the presence of other sediment constituents. To put this in better perspective, the sediment constituents are now discussed individually with reference to the accompanying figures.

Red Blood Cells

In the urine, RBCs appear as smooth, non-nucleated, biconcave disks measuring approximately 7 mm in diameter (Fig. 6-8). They must be identified using high-power (40×) objective (×400 magnification). RBCs are routinely reported as the average number seen in 10 hpfs.

In concentrated (hypersthenuric) urine, the cells shrink due to loss of water and may appear *crenated* or irregularly shaped (Fig. 6-9). In dilute (hyposthenuria) urine, the cells absorb water, swell, and lyse rapidly, releasing their hemoglobin and leaving only the cell membrane. These large empty cells are called *ghost cells* and can be easily missed if specimens are not examined under reduced light.

Of all the sediment elements, RBCs are the most difficult for students to recognize. The reasons for this include RBCs' lack of characteristic structures, variations in size,



Figure 6-9 Microcytic and crenated RBCs (×400).



Figure 6–10 Yeast. The presence of budding forms aid in distinguishing from RBCs (×400).

and close resemblance to other sediment constituents. RBCs are frequently confused with yeast cells, oil droplets, and air bubbles. Yeast cells usually exhibit budding (Fig. 6-10). Oil droplets and air bubbles are highly refractile when the fine adjustment is focused up and down (Fig. 6-11); they may also appear in a different plane than other sediment constituents (Fig. 6-12). The rough appearance of crenated RBCs may resemble the granules seen in WBCs; however, they are much smaller than WBCs. Should the identification continue to be doubtful, adding acetic acid to a portion of the sediment will lyse the RBCs, leaving the yeast, oil droplets, and WBCs intact. Supravital staining may also be helpful.

Studies have focused on the morphology of urinary RBCs as an aid in determining the site of renal bleeding. RBCs that vary in size, have cellular protrusions, or are fragmented are termed *dysmorphic* (Fig. 6-13) and have been associated primarily with glomerular bleeding. The number and appearance of the dysmorphic cells must also be considered, because abnormal urine concentration affects RBC appearance, and small numbers of dysmorphic cells are found with non-glomerular hematuria. ^{10,11} Dysmorphic RBCs also have been demonstrated after strenuous exercise, indicating a glomeru-

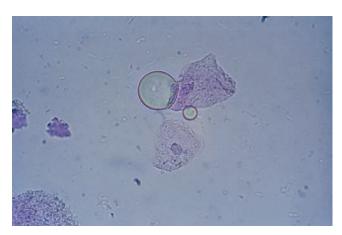


Figure 6–11 KOVA-stained squamous epithelial cells and oil droplets (×400).

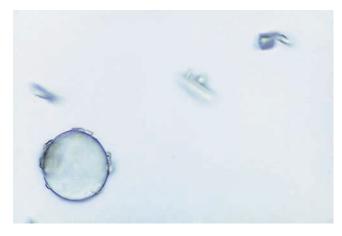


Figure 6–12 Air bubble. Notice no formed elements are in focus (\times 100).

lar origin of this phenomenon.¹² The dysmorphic cell most closely associated with glomerular bleeding appears to be the acanthocyte with multiple protrusions, which may be difficult to observe under bright-field microscopy.^{13,14} Further analysis of sediments containing dysmorphic RBCs using Wright's stained preparations shows the cells to be hypochromic and better delineates the presence of cellular blebs and protrusions.

Clinical Significance

The presence of RBCs in the urine is associated with damage to the glomerular membrane or vascular injury within the genitourinary tract. The number of cells present is indicative of the extent of the damage or injury. Patient histories often mention the presence of macroscopic versus microscopic hematuria.

When macroscopic hematuria is present, the urine appears cloudy with a red to brown color. Microscopic analysis may be reported in terms of greater than 100 per hpf or as specified by laboratory protocol. Macroscopic hematuria is frequently associated with advanced glomerular damage but is also seen with damage to the vascular integrity of the uri-

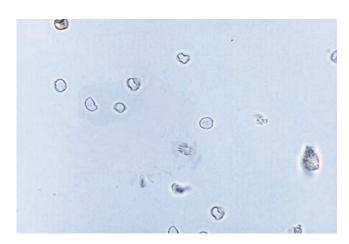


Figure 6–13 Dysmorphic RBCs (×400).

94

nary tract caused by trauma, acute infection or inflammation, and coagulation disorders.

The observation of microscopic hematuria can be critical to the early diagnosis of glomerular disorders and malignancy of the urinary tract and to confirm the presence of renal calculi. The presence of not only RBCs but also hyaline, granular, and RBC casts may be seen following strenuous exercise. These abnormalities are nonpathologic and disappear after rest. 15 The possibility of menstrual contamination must also be considered in specimens from female patients.

As discussed previously, the presence or absence of RBCs in the sediment cannot always be correlated with specimen color or a positive chemical test result for blood. The presence of hemoglobin that has been filtered by the glomerulus produces a red urine with a positive chemical test result for blood in the absence of microscopic hematuria. Likewise, a specimen appearing macroscopically normal may contain a small but pathologically significant number of RBCs when examined microscopically.

White Blood Cells

WBCs are larger than RBCs, measuring an average of about 12 mm in diameter (Fig. 6-14).

The predominant WBC found in the urine sediment is the neutrophil. Neutrophils are much easier to identify than RBCs because they contain granules and multilobed nuclei (Figs. 6-15 and 6-16). However, they are still identified using high power microscopy and are also reported as the average number seen in 10 hpfs. Neutrophils lyse rapidly in dilute alkaline urine and begin to lose nuclear detail.

Neutrophils exposed to hypotonic urine absorb water and swell. Brownian movement of the granules within these larger cells produces a sparkling appearance, and they are

referred to as "glitter cells." When stained with Sternheimer-Malbin stain, these large cells stain light blue as opposed to the violet color usually seen with neutrophils. They are of no pathologic significance.

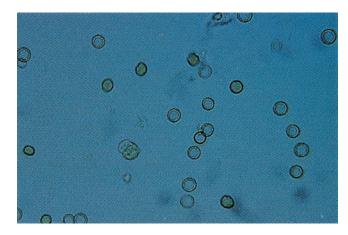


Figure 6–14 RBCs and one WBC (\times 400).

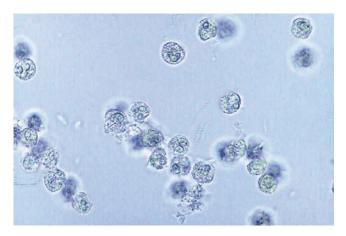
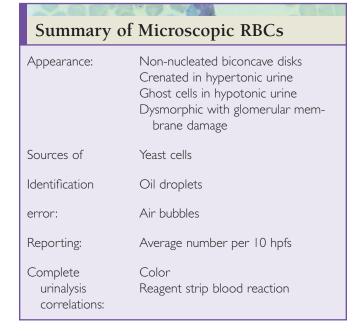


Figure 6–15 WBCs. Notice the multilobed nucleoli (×400).



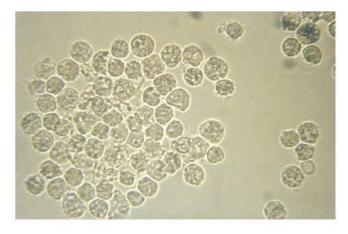


Figure 6–16 WBC clump (×400).

Eosinophils

The presence of urinary eosinophils is primarily associated with drug-induced interstitial nephritis; however, small numbers of eosinophils may be seen with urinary tract infection (UTI) and renal transplant rejection. Evaluation of a concentrated, stained urine sediment is required for performing a urinary eosinophil test. Sediment may be concentrated by routine centrifugation alone or with cytocentrifugation. The preferred eosinophil stain is Hansel (Fig. 6-17); however, Wright's stain can also be used. The percentage of eosinophils in 100 to 500 cells is determined. Eosinophils are not normally seen in the urine; therefore, the finding of more than 1% eosinophils is considered significant. ¹⁶

Mononuclear Cells

Lymphocytes, monocytes, macrophages, and histiocytes may be present in small numbers and are usually not identified in the wet preparation urine microscopic analysis. Because lymphocytes are the smallest WBCs, they may resemble RBCs. They may be seen in increased numbers in the early stages of renal transplant rejection. Monocytes, macrophages, and histiocytes are large cells and may appear vacuolated or contain inclusions. Specimens containing an increased amount of mononuclear cells that cannot be identified as epithelial cells should be referred for cytodiagnostic urine testing.

The primary concern in the identification of WBCs is the differentiation of mononuclear cells and disintegrating neutrophils from renal tubular epithelial (RTE) cells. RTE cells are usually larger than WBCs and more polyhedral in shape, with an eccentrically located nucleus. WBCs in the process of ameboid motion may be difficult to distinguish from epithelial cells because of their irregular shape. Supravital staining or the addition of acetic acid can be used to enhance nuclear detail (Fig. 6-18), if necessary.

Usually, fewer than five leukocytes per hpf are found in normal urine; however, higher numbers may be present in urine from females. Although leukocytes, like RBCs, may enter the urine through glomerular or capillary trauma, they are also capable of ameboid migration through the tissues to

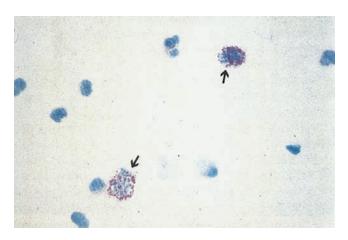


Figure 6–17 Hansel-stained eosinophils (×400).

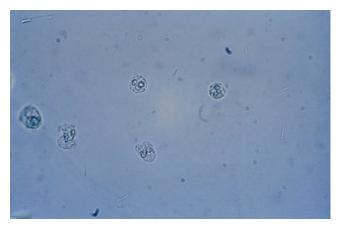


Figure 6–18 WBCs with acetic acid nuclear enhancement (×400).

sites of infection or inflammation. An increase in urinary WBCs is called *pyuria* and indicates the presence of an infection or inflammation in the genitourinary system. Bacterial infections, including pyelonephritis, cystitis, prostatitis, and urethritis, are frequent causes of pyuria. However, pyuria is also present in nonbacterial disorders, such as glomerulonephritis, lupus erythematosus, interstitial nephritis, and tumors. Reporting the presence of bacteria in specimens containing leukocytes is important.

Epithelial Cells

It is not unusual to find epithelial cells in the urine, because they are derived from the linings of the genitourinary system. Unless they are present in large numbers or in abnormal forms, they represent normal sloughing of old cells. Three types of epithelial cells are seen in urine: squamous, transitional (urothelial), and renal tubular (Fig. 6-19). They are

Summary of Microscopic WBCs			
Appearance:	Larger than RBCs Granulated, multilobed neutrophils Glitter cells in hypotonic urine Mononuclear cells with abundant cytoplasm		
Sources of identification error:	Renal tubular epithelial cells		
Reporting:	Average number per 10 hpfs		
Complete urinalysis correlations:	Leukocyte esterase Nitrite Specific gravity pH		



Figure 6–19 Sediment-containing squamous, caudate transitional, and RTE cells (×400).

classified according to their site of origin within the genitourinary system.

Squamous Epithelial Cells

Squamous cells are the largest cells found in the urine sediment. They contain abundant, irregular cytoplasm and a prominent nucleus about the size of an RBC (Figs. 6-20 and 6-21). They are often the first structures observed when the sediment is examined under low-power magnification. Usually at least a few squamous epithelial cells are present in the sediment and can serve as a good reference for focusing of the microscope (Fig. 6-22). After examination of the appropriate number of fields, squamous epithelial cells are commonly reported in terms of rare, few, moderate, or many. They are reported in terms of low-power or high-power magnification based on laboratory protocol.

Difficulty identifying squamous cells is rare. However, they may occasionally appear folded, possibly resembling a cast, and will begin to disintegrate in urine that is not fresh. In sediments containing large amounts of squamous cells, it may be more difficult to enumerate smaller pathologic elements, such as RBCs and WBCs, and they should be carefully examined (Figs. 6-23 and 6-24).

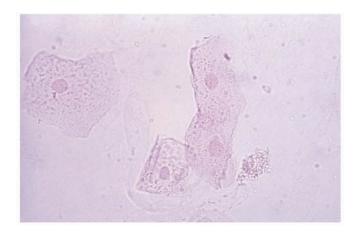


Figure 6–20 KOVA-stained squamous epithelial cells (×400).

Squamous epithelial cells originate from the linings of the vagina and female urethra and the lower portion of the male urethra. They represent normal cellular sloughing and have no pathologic significance. Increased amounts are more

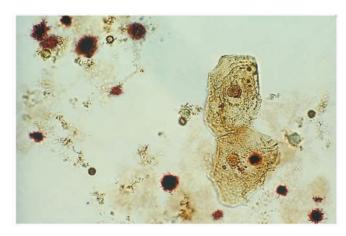


Figure 6–21 Phenazopyridine-stained sediment showing squamous epithelial cells and phenazopyridine crystals formed following refrigeration (×400).

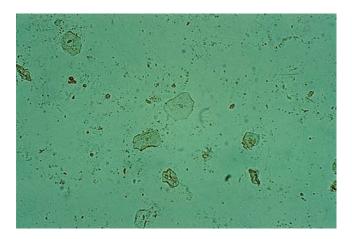


Figure 6–22 Squamous epithelial cells identifiable under low power $(\times 100)$.

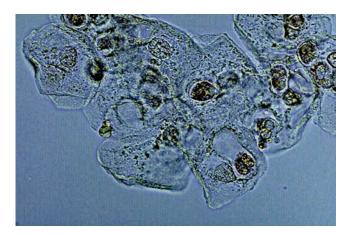


Figure 6–23 Clump of squamous epithelial cells (×400).



Figure 6–24 Clump of squamous epithelial cells with folded forms (×400).

frequently seen in urine from female patients. Specimens collected using the midstream clean-catch technique contain less squamous cell contamination.

A variation of the squamous epithelial cell is the *clue cell*, which does have pathologic significance. Clue cells are indicative of vaginal infection by the bacterium *Gardnerella vaginalis*. They appear as squamous epithelial cells covered with the *Gardnerella* coccobacillus. To be considered a clue cell, the bacteria should cover most of the cell surface and extend beyond the edges of the cell. This gives the cell a granular, irregular appearance. Routine testing for clue cells is performed by examining a vaginal wet preparation for the presence of the characteristic cells. However, small numbers of clue cells may be present in the urinary sediment. Microscopists should remain alert for their presence, as urinalysis may be the first test performed on the patient.

Transitional Epithelial (Urothelial) Cells

Transitional epithelial cells are smaller than squamous cells and appear in several forms, including spherical, polyhedral, and caudate (Figs. 6-25 and 6-26). These differences are caused by the ability of transitional epithelial cells to absorb large amounts of water. Cells in direct contact with the urine absorb water, becoming spherical in form and much larger than the polyhedral and caudate cells. All forms have distinct, centrally located nuclei. Transitional cells are identified and enumerated using high-power magnification. Like squamous cells, they are usually reported as rare, few, moderate, or many following laboratory protocol.

Spherical forms of transitional epithelial cells are sometimes difficult to distinguish from RTE cells. The presence of a centrally located rather than eccentrically placed nucleus, and supravital staining, can aid in the differentiation.

Transitional epithelial cells originate from the lining of the renal pelvis, calyces, ureters, and bladder, and from the upper portion of the male urethra. They are usually present in small numbers in normal urine, representing normal cellular sloughing. Increased numbers of transitional cells seen singly, in pairs, or in clumps (*syncytia*) are present following inva-

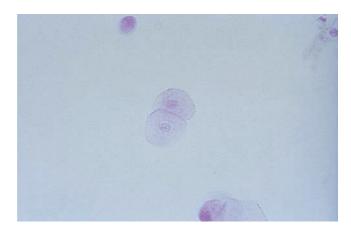


Figure 6–25 KOVA-stained spherical transitional epithelial cells $(\times 400)$.

sive urologic procedures such as catheterization and are of no clinical significance (Fig. 6-27). An increase in transitional cells exhibiting abnormal morphology such as vacuoles and irregular nuclei may be indicative of malignancy or viral infection. In such cases, the specimen should be referred for cytologic examination.

Renal Tubular Epithelial Cells

Renal tubular epithelial (RTE) cells vary in size and shape depending on the area of the renal tubules from which they originate. The cells from the proximal convoluted tubule (PCT) are larger than other RTE cells. They tend to have a rectangular shape and are referred to as columnar or convoluted cells. The cytoplasm is coarsely granular, and the RTE cells often resemble casts. They should be closely examined for the presence of a nucleus, as a nucleus would not be present in a cast. Notice the nucleus and granules in Figure 6-28. This is a PCT cell that has absorbed fat globules and could easily be mistaken for a granular or fatty cast.

Cells from the distal convoluted tubule (DCT) are smaller than those from the PCT and are round or oval. They can be mistaken for WBCs and spherical transitional epithe-



Figure 6–26 Caudate transitional epithelial cells (×400).

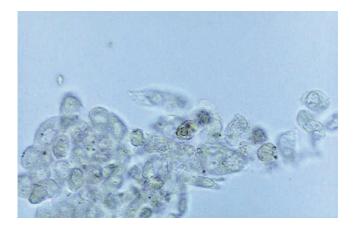


Figure 6-27 Syncytia of transitional epithelial cells (×400).

lial cells. Observation of the eccentrically placed round nucleus aids in differentiating them from spherical transitional cells (Fig. 6-29).

Collecting duct RTE cells are cuboidal and are never round. Along with the eccentrically placed nucleus, the presence of at least one straight edge differentiates them from spherical and polyhedral transitional cells (Fig. 6-30). Because RTE cells are often present as a result of tissue destruction (necrosis), the nucleus is not easily visible in unstained sediment.

Cells from the collecting duct that appear in groups of three or more are called renal fragments. They are frequently seen as large sheets of cells. PCT and DCT cells are not seen in large sheets of cells (Fig. 6-31).

RTE cells must be identified and enumerated using high-power magnification. Depending on laboratory protocol, they may be reported as rare, few, moderate, or many, or as the actual number per high-power field. Classification of RTE cells as to site of origin is not considered a part of the routine sediment analysis and often requires special staining techniques. The presence of more than two RTE cells per high-power field indicates tubular injury, and such specimens should be referred for cytologic urine testing. ¹⁷



Figure 6–28 RTE cell. Columnar proximal convoluted tubule cell with granules and attached fat globules (×400).

Clinical Significance

RTE cells are the most clinically significant of the epithelial cells. The presence of increased amounts is indicative of necrosis of the renal tubules, with the possibility of affecting overall renal function.

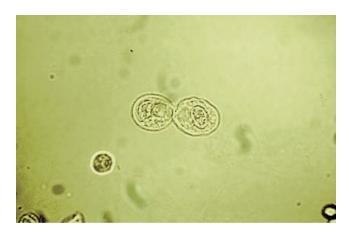


Figure 6–29 RTE cells. Oval distal convoluted tubule cells. Notice the eccentrically placed nuclei (×400).

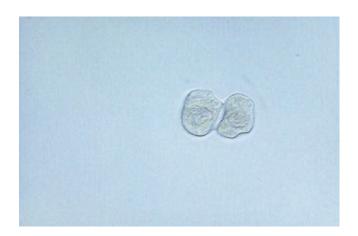


Figure 6–30 RTE cells, cuboidal from the collecting duct $(\times 400)$.

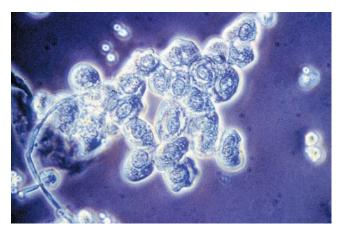


Figure 6–31 Fragment of RTE cells under phase microscopy (×400).

Conditions producing tubular necrosis include exposure to heavy metals, drug-induced toxicity, hemoglobin and myoglobin toxicity, viral infections (hepatitis B), pyelonephritis, allergic reactions, malignant infiltrations, salicylate poisoning, and acute allogenic transplant rejection. RTE cells may also be seen as secondary effects of glomerular disorders. Renal fragments are an indication of severe tubular injury with basement membrane disruption. Single cuboidal cells are particularly noticeable in cases of salicylate poisoning:.

Because one of the functions of RTE cells is reabsorption of the glomerular filtrate, it is not unusual for them to contain substances from the filtrate. RTE cells absorb bilirubin present in the filtrate as the result of liver damage, such as occurs with viral hepatitis, and appear a deep yellow color. As discussed in Chapter 5, hemoglobin present in the filtrate is absorbed by the RTE cells and converted to hemosiderin. Therefore, following episodes of hemoglobinuria (transfusion reactions, paroxysmal nocturnal hemoglobinuria, etc.), the RTE cells may contain the characteristic yellow-brown hemosiderin granules. The granules may also be seen free-floating in the sediment. Confirmation of the presence of hemosiderin is performed by staining the sediment with Prussian blue. The iron-containing hemosiderin granules stain blue (Fig. 6-32).

Oval Fat Bodies

RTE cells absorb lipids that are present in the glomerular filtrate. They then appear highly refractile, and the nucleus may be more difficult to observe. These lipid-containing RTE cells are called oval fat bodies (Fig. 6-33). They are usually seen in conjunction with free-floating fat droplets.

Identification of oval fat bodies is confirmed by staining the sediment with Sudan III or Oil Red O fat stains and examining the sediment using polarized microscopy. The droplets are composed of triglycerides, neutral fats, and cholesterol. Fat stains stain triglycerides and neutral fats, producing orange-red droplets (Fig. 6-34). Examination of the sediment using polarized light results in the appearance of characteristic Maltese cross formations in droplets containing cholesterol

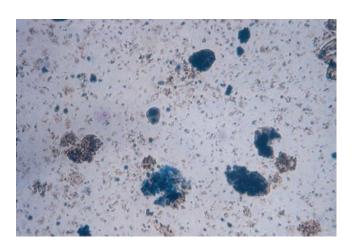


Figure 6-32 Prussian blue-stained hemosiderin granules.

(Fig. 6-35). Sediments negative for fat after staining should still be checked using polarized light in case only cholesterol is present. Likewise, staining should be performed on sediments negative under polarized light. Oval fat bodies are reported as the average number per hpf.

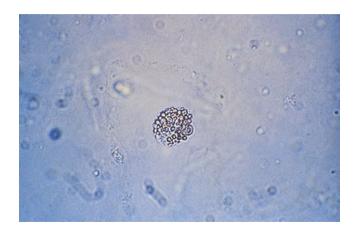


Figure 6–33 Oval fat body (\times 400).

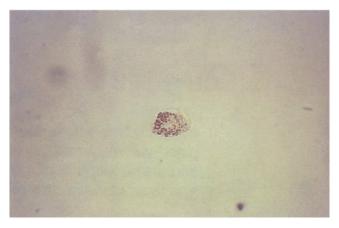


Figure 6-34 Sudan III-stained oval fat body (×400).

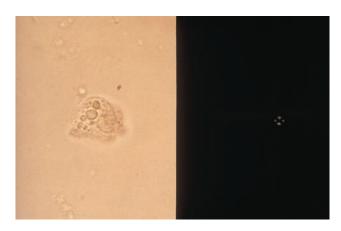


Figure 6–35 Oval fat body under bright-field and polarized microscopy. Notice the Maltese cross formation (×400).

Free-floating fat droplets also stain or polarize depending on their composition. They may be observed floating on the top of the specimen. Care should be taken not to confuse the droplets with starch and crystal particles that also polarize. Specimen contamination by vaginal preparations and lubricants used in specimen collection must be considered when only free-floating fat droplets are present.

Lipiduria is most frequently associated with damage to the glomerulus caused by the nephrotic syndrome (see Chapter 8). It is also seen with severe tubular necrosis, diabetes mellitus, and in trauma cases that cause release of bone marrow fat from the long bones. In lipid-storage diseases, large fat-laden histiocytes may also be present. They can be differentiated from oval fat bodies by their large size.

In cases of acute tubular necrosis, RTE cells containing large, nonlipid-filled vacuoles may be seen along with normal renal tubular cells and oval fat bodies. Referred to as "bubble cells," they appear to represent injured cells in which the endoplasmic reticulum has dilated prior to cell death. ¹⁸

Bacteria

Bacteria are not normally present in urine. However, unless specimens are collected under sterile conditions (catheterization), a few bacteria are usually present as a result of vaginal, urethral, external genitalia, or collection-container contamination. These contaminant bacteria multiply rapidly in specimens that remain at room temperature for extended periods, but are of no clinical significance. They may produce a positive nitrite test result and also frequently result in a pH above 8, indicating an unacceptable specimen.

Bacteria may be present in the form of cocci (spherical) or bacilli (rods). Owing to their small size, they must be observed and reported using high-power magnification. They are reported as few, moderate, or many per high-power field. To be considered significant for UTI, bacteria should be accompanied by WBCs. Some laboratories report bacteria only when observed in fresh specimens in conjunction with WBCs (Fig. 6-36). The presence of motile organisms in a drop of fresh urine collected under sterile conditions correlates well with a positive urine culture. Observing bacteria for

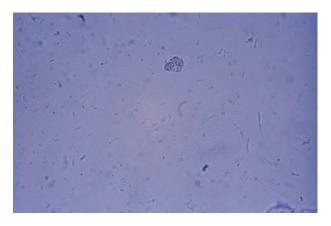


Figure 6–36 KOVA-stained bacteria and WBC (×400).

motility also is useful in differentiating them from similarly appearing amorphous phosphates and urates. Use of phase microscopy aids in the visualization of bacteria.

The presence of bacteria can be indicative of either lower or upper UTI. Specimens containing increased bacteria and leukocytes are routinely followed up with a specimen for quantitative urine culture. The bacteria most frequently associated with UTI are the Enterobacteriaceae (referred to as gram-negative rods); however, the cocci-shaped *Staphylococcus* and *Enterococcus* are also capable of causing UTI. The actual bacteria producing an UTI cannot be identified with the microscopic examination.

Yeast

Yeast cells appear in the urine as small, refractile oval structures that may or may not contain a bud. In severe infections, they may appear as branched, mycelial forms (Fig. 6-37). Yeast cells are reported as rare, few, moderate, or many per hpf.

Differentiation between yeast cells and RBCs can sometimes be difficult. Careful observation for budding yeast cells should be helpful, as shown in Figure 6-10.

Yeast cells, primarily *Candida albicans*, are seen in the urine of diabetic, immunocompromised patients and women with vaginal moniliasis. The acidic, glucose-containing urine of patients with diabetes provides an ideal medium for the growth of yeast. As with bacteria, a small amount of yeast entering a specimen as a contaminant multiplies rapidly if the specimen is not examined while fresh. A true yeast infection should be accompanied by the presence of WBCs.

Parasites

The most frequent parasite encountered in the urine is *Trichomonas vaginalis*. The *Trichomonas* trophozoite is a pear-shaped flagellate with an undulating membrane. It is easily identified in wet preparations of the urine sediment by its rapid darting movement in the microscopic field. *Trichomonas* is usually reported as rare, few, moderate, or many per hpf.

When not moving, *Trichomonas* is more difficult to identify and may resemble a WBC, transitional, or RTE cell. Use of phase microscopy may enhance visualization of the flagella or undulating membrane.

T. vaginalis is a sexually transmitted pathogen associated primarily with vaginal inflammation. Infection of the male urethra and prostate is asymptomatic.

The ova of the bladder parasite *Schistosoma haematobium* will appear in the urine. However, this parasite is seldom seen in the United States.

Fecal contamination of a urine specimen can also result in the presence of ova from intestinal parasites in the urine sediment. The most common contaminant is ova from the pinworm *Enterobius vermicularis*.

Spermatozoa

Spermatozoa are easily identified in the urine sediment by their oval, slightly tapered heads and long, flagellalike tails (Fig. 6-38). Urine is toxic to spermatozoa; therefore,

Summary of	Epithelial Cells		
Squamous Cells		RTE Cells	
Appearance:	Largest cells in the sediment with abundant, irregular cytoplasm and prominent nucleii	Appearance:	Rectangular, columnar, round, oval or, cuboidal with an eccentric nucleus possibly bilirubin-stained or hemosiderin-laden
Sources of error:	Rarely encountered, folded cells may resemble casts	Sources of error:	Spherical transitional cells Granular casts
Reporting:	Rare, few, moderate, or many per lpf		Granular Casis
Complete	Clarity	Reporting:	Average number per 10 hpfs
urinalysis correlations:		Complete urinalysis	Leukocyte esterase and nitrite (pyelonephritis)
Transitional Cells		correlations:	Color Clarity
Appearance:	Spherical, polyhedral, or caudate with centrally located nucleus		Protein Bilirubin (hepatitis) Blood
Sources of error:	Spherical forms resemble RTE cells	Oval Fat Bodies	
Reporting:	Rare, few, moderate, or many per hpf	Appearance:	Highly refractile RTE cells
Complete urinalysis correlations:	Clarity; blood, if malignancy-associated	Sources of error:	Confirm with fat stains and polarized microscopy
		Reporting:	Average number per hpf
		Complete urinalysis correlations:	Clarity Blood Protein Free fat droplets/fatty casts

they rarely exhibit the motility observed when examining a semen specimen.

Spermatozoa are occasionally found in the urine of both men and women following sexual intercourse, masturbation, or nocturnal emission. They are rarely of clinical significance except in cases of male infertility or retrograde ejaculation in which sperm is expelled into the bladder instead of the urethra. A positive reagent strip test for protein may be seen when increased amounts of semen are present.

Laboratory protocols vary with regard to reporting or not reporting the presence of spermatozoa in a urine specimen. Laboratories not reporting its presence cite the lack of clinical significance and possible legal consequences. Laboratories supporting the reporting of spermatozoa cite the possible clinical significance and the minimal possibility of legal consequences. ¹⁹



Figure 6–37 Yeast showing mycelial forms (×400).

Figure 6–38 Spermatozoa (×400).

Mucus

Mucus is a protein material produced by the glands and epithelial cells of the lower genitourinary tract and the RTE cells. Immunologic analysis has shown that *Tamm-Horsfall protein* is a major constituent of mucus.

Mucus appears microscopically as thread-like structures with a low refractive index (Fig. 6-39). Subdued light is required when using bright-field microscopy. Care must be taken not to confuse clumps of mucus with hyaline casts. The differentiation can usually be made by observing the irregular appearance of the mucous threads. Mucous threads are reported as rare, few, moderate, or many per lpf.

Mucus is more frequently present in female urine specimens. It has no clinical significance when present in either female or male urine.

Casts

Casts are the only elements found in the urinary sediment that are unique to the kidney. They are formed within the lumens of the distal convoluted tubules and collecting ducts, providing a microscopic view of conditions within the

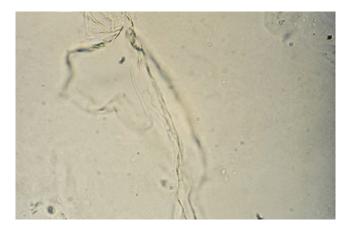


Figure 6–39 Mucus threads (×400).

nephron. Their shape is representative of the tubular lumen, with parallel sides and somewhat rounded ends, and they may contain additional elements present in the filtrate.

Examination of the sediment for the detection of casts is performed using lower power magnification. When the glass cover-slip method is used, low-power scanning should be performed along the edges of the cover slip. Observation under subdued light is essential, because the cast matrix has a low refractive index. Similar to many other sediment constituents, the cast matrix dissolves quickly in dilute, alkaline urine. Once detected, casts must be further identified as to composition using high-power magnification. They are reported as the average number per 10 lpfs.

Cast Composition and Formation

The major constituent of casts is Tamm-Horsfall protein, a glycoprotein excreted by the RTE cells of the distal convoluted tubules and upper collecting ducts. Other proteins present in the urinary filtrate, such as albumin and immunoglobulins, are also incorporated into the cast matrix. Under normal conditions, Tamm-Horsfall protein is excreted at a relatively constant rate. The rate of excretion appears to increase under conditions of stress and exercise, which may account for the transient appearance of hyaline casts when these conditions are present. The protein gels more readily under conditions of urine-flow stasis, acidity, and the presence of sodium and calcium. The extent of protein glycosylation is also important.20 Tamm-Horsfall protein is found in both normal and abnormal urine and, as discussed previously, is a major constituent of mucus. It is not detected by reagent strip protein methods. Therefore, the increased urinary protein frequently associated with the presence of casts is caused by underlying renal conditions.

Scanning electron microscope studies have provided a step-by-step analysis of the formation of the Tamm-Horsfall protein matrix²¹:

- 1. Aggregation of Tamm-Horsfall protein into individual protein fibrils attached to the RTE cells
- 2. Interweaving of protein fibrils to form a loose fibrillar network (urinary constituents may become enmeshed in the network at this time)
- 3. Further protein fibril interweaving to form a solid structure
- 4. Possible attachment of urinary constituents to the solid matrix
- 5. Detachment of protein fibrils from the epithelial cells
- 6. Excretion of the cast

As the cast forms, urinary flow within the tubule decreases as the lumen becomes blocked. The accompanying dehydration of the protein fibrils and internal tension may account for the wrinkled and convoluted appearance of older hyaline casts.²² The width of the cast depends on the size of the tubule in which it is formed. Broad casts may result from

Summary of	Miscellaneous Structures		
Bacteria		Reporting:	Rare, few, moderate, or many per hpf
Appearance:	Small spherical and rod-shaped structures	Complete urinalysis	LE WBCs
Sources of error:	Amorphous phosphates and urates	correlations:	
Reporting:	Few, moderate, or many per hpf, the presence of WBCs may be	Spermatozoa	
	required	Appearance:	Tapered oval head with long, thin tail
Complete urinalysis correlations:	pH Nitrite LE WBCs	Sources of error:	None
Yeast		Reporting:	Present, based on laboratory protocol
Appearance:	Small, oval, refractile structures with buds and/or mycelia	Complete urinalysis correlations:	Protein
Sources of error:	RBCs	Mucus	
Reporting:	Rare, few, moderate, or many per hpf,	racus	
	the presence of WBCs may be required	Appearance:	Single or clumped threads with a low refractive index
Complete urinalysis correlations:	Glucose LE WBCs	Sources of error:	Hyaline casts
Trichomonas		Reporting:	Rare, few, moderate, or many per lpf
Appearance:	Pear-shaped, motile, flagellated	Complete urinalysis	None
Sources of error:	WBCs, renal tubular epithelial cells	correlations:	

tubular distention or, in the case of extreme urine stasis, from formation in the collecting ducts. Formation of casts at the junction of the ascending loop of Henle and the distal convoluted tubule may produce structures with a tapered end. These have been referred to as cylindroids, but they have the same significance as casts. In fact, the presence of urinary casts is termed *cylindruria*. The appearance of a cast is also influenced by the materials present in the filtrate at the time of its formation and the length of time it remains in the tubule. Any elements present in the tubular filtrate, including cells, bacteria, granules, pigments, and crystals, may become embedded in or attached to the cast matrix. The types of casts found in the sediment represent different clinical conditions and will be discussed separately in this section.

Hyaline Casts

The most frequently seen cast is the hyaline type, which consists almost entirely of Tamm-Horsfall protein. The presence of zero to two hyaline casts per lpf is considered normal, as is the finding of increased numbers following strenuous exercise, dehydration, heat exposure, and emotional stress. ¹⁵ Pathologically, hyaline casts are increased in acute glomerulonephritis, pyelonephritis, chronic renal disease, and congestive heart failure.

Hyaline casts appear colorless in unstained sediments and have a refractive index similar to that of urine; thus, they can easily be overlooked if specimens are not examined under subdued light (Figs. 6-40 and 6-41). Sternheimer-

Malbin stain produces a pink color in hyaline casts. Increased visualization can be obtained by phase microscopy (Figs. 6-42 and 6-43).

The morphology of hyaline casts is varied, consisting of normal parallel sides and rounded ends, cylindroid forms, and wrinkled or convoluted shapes that indicate aging of the cast matrix (Fig. 6-44). The presence of an occasional adher-



Figure 6–40 Hyaline cast under low power with amorphous granules and mucus (×100).



Figure 6–41 Hyaline cast and amorphous urates attached to mucus pseudocast.

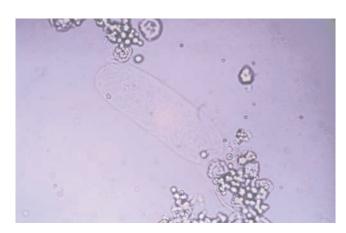


Figure 6–42 Hyaline cast (×400).

ing cell or granule may also be observed (Fig. 6-45) but does not change the cast classification.

RBC Casts

Whereas the finding of RBCs in the urine indicates bleeding from an area within the genitourinary tract, the presence of

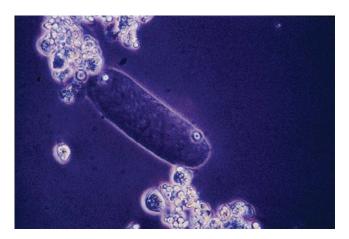


Figure 6–43 Hyaline cast under phase microscopy (×400).



Figure 6-44 Convoluted hyaline cast (×400).



Figure 6–45 Hyaline cast containing occasional granules (×400).

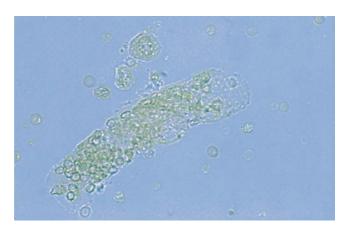


Figure 6–46 RBC cast. Notice the presence of hypochromic and dysmorphic free RBCs (×400).

RBC casts is much more specific, showing bleeding within the nephron. RBC casts are primarily associated with damage to the glomerulus (glomerulonephritis) that allows passage of the cells through the glomerular membrane; however, any damage to the nephron capillary structure can cause their formation. RBC casts associated with glomerular damage are usually associated with proteinuria and dysmorphic erythrocytes. RBC casts have also been observed in healthy individuals following participation in strenuous contact sports. ¹⁵

RBC casts are easily detected under low power by their orange-red color. They are more fragile than other casts and may exist as fragments or have a more irregular shape as the result of tightly packed cells adhering to the protein matrix (Figs. 6-46 and 6-47). Examination under high-power magnification should concentrate on determining that a cast matrix is present, thereby differentiating the structure from a clump of RBCs. Because of the serious diagnostic implications of RBC casts, the actual presence of RBCs must also be verified to prevent the inaccurate reporting of nonexistent RBC casts. It is highly improbable that RBC casts will be present in the absence of free-standing RBCs and a positive reagent strip test for blood (Fig. 6-48).

As an RBC cast ages, cell lysis begins and the cast develops a more homogenous appearance, but retains the charac-

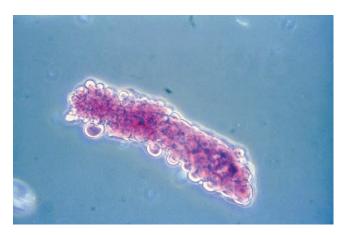


Figure 6–47 KOVA-stained RBC cast under phase microscopy (×400).

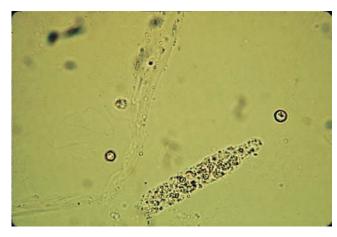


Figure 6–48 Disintegrating RBC cast. Notice the presence of free RBCs to confirm identification.

teristic orange-red color from the released hemoglobin (Fig. 6-49). These casts may be distinguished as blood casts, indicating greater stasis of urine flow. However, because all casts containing blood have the same clinical significance, this is not considered necessary. Both types of casts are reported as the number of RBC casts per lpf.

In the presence of massive hemoglobinuria or myoglobinuria, homogenous orange-red or red-brown casts may be observed. Granular, dirty, brown casts representing hemoglobin degradation products such as methemoglobin may also be present (Fig. 6-50). They are associated with the acute tubular necrosis often caused by the toxic effects of massive hemoglobinuria that can lead to renal failure. These dirty, brown casts must be present in conjunction with other pathologic findings such as RTE cells and a positive reagent strip test for blood.

WBC Casts

The appearance of WBC casts in the urine signifies infection or inflammation within the nephron. They are most frequently associated with pyelonephritis and are a primary marker for distinguishing pyelonephritis (upper UTI) from lower UTIs. However, they are also present in nonbacterial



Figure 6–49 Cast containing hemoglobin pigment. A comparison of RBCs and yeast also can be made (×400).

Figure 6–50 Granular, dirty brown cast (×400).

inflammations such as acute interstitial nephritis and may accompany RBC casts in glomerulonephritis.

WBC casts are visible under low-power magnification but must be positively identified using high power. Most frequently, WBC casts are composed of neutrophils; therefore, they may appear granular, and, unless disintegration has occurred, multilobed nuclei will be present (Fig. 6-51). Supravital staining may be necessary to demonstrate the characteristic nuclei (Fig. 6-52). It is particularly helpful for differentiating WBC casts from RTE casts. Observation of free WBCs in the sediment is also essential. Bacteria are present in cases of pyelonephritis, but are not present with acute interstitial nephritis; however, eosinophil casts may be present in appropriately stained specimens.

Casts tightly packed with WBCs may have irregular borders. These structures should be carefully examined to determine that a cast matrix is present. WBCs frequently form clumps, and these do not have the same significance as casts (Fig. 6-53).

Bacterial Casts

Bacterial casts containing bacilli both within and bound to the protein matrix are seen in pyelonephritis.²³ They may be pure bacterial casts or mixed with WBCs.

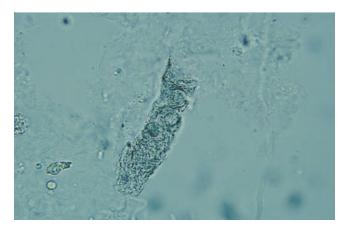


Figure 6–51 Disintegrating WBC cast (×400).

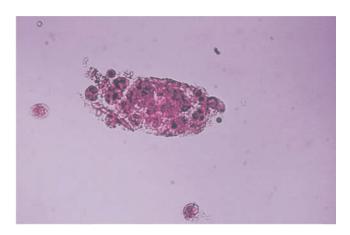


Figure 6–52 KOVA-stained WBC cast (×400).

Identification of bacterial casts can be difficult, because packed casts packed with bacteria can resemble granular casts. Their presence should be considered when WBC casts and many free WBCs and bacteria are seen in the sediment. Confirmation of bacterial casts is best made by performing a Gram stain on the dried or cytocentrifuged sediment.

Epithelial Cell Casts

Casts containing RTE cells represent the presence of advanced tubular destruction, producing urinary stasis along with disruption of the tubular linings. Similar to RTE cells, they are associated with heavy metal and chemical or drug-induced toxicity, viral infections, and allograft rejection. They also accompany WBC casts in cases of pyelonephritis.

As discussed previously, the fibrils of Tamm-Horsfall protein that make up the cast matrix remain attached to the RTE cells that produce them; therefore, the observation of an occasional tubular cell attached to a hyaline cast can be expected. When tubular damage is present, some cells may be incorporated into the cast matrix, but the majority will be very noticeably attached to the cast surface.

Owing to the formation of casts in the distal convoluted tubule, the cells visible on the cast matrix are the smaller, round, and oval cells (Fig. 6-54). They may be difficult to



Figure 6–53 WBC clump. Notice the absence of a cast matrix.

differentiate from WBCs, particularly if degeneration has occurred. Staining and the use of phase microscopy can be helpful to enhance the nuclear detail needed for identification (Figs. 6-55 and 6-56). Fragments of epithelial tissue may also be attached to the cast matrix. Bilirubin-stained RTE cells are seen in cases of hepatitis (Fig. 6-57).

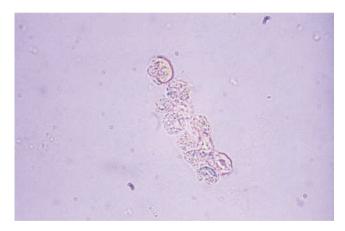


Figure 6-54 RTE cell cast (×400).

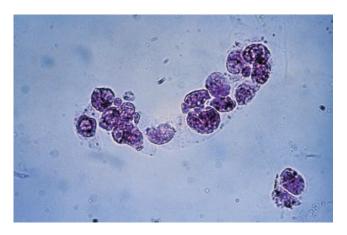


Figure 6–55 KOVA-stained RTE cell cast (×400).

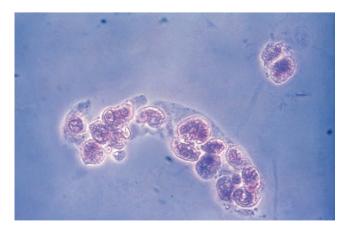


Figure 6–56 KOVA-stained RTE cell cast under phase microscopy (×400).

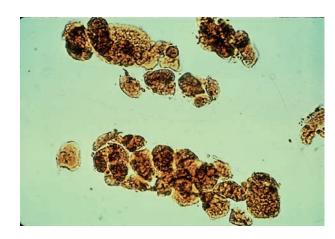


Figure 6–57 RTE cast with bilirubin stained cells (×400).

Fatty Casts

Fatty casts are seen in conjunction with oval fat bodies and free fat droplets in disorders causing lipiduria. They are most frequently associated with the nephrotic syndrome, but are also seen in toxic tubular necrosis, diabetes mellitus, and crush injuries.

Fatty casts are highly refractile under bright-field microscopy. The cast matrix may contain few or many fat droplets, and intact oval fat bodies may be attached to the matrix (Figs. 6-58 to 6-60). Confirmation of fatty casts is performed using polarized microscopy and Sudan III or Oil Red O fat stains. As discussed previously, cholesterol demonstrates characteristic Maltese cross formations under polarized light, and triglycerides and neutral fats stain orange with fat stains. Fats do not stain with Sternheimer-Malbin stains.

Mixed Cellular Casts

Considering that a variety of cells may be present in the urinary filtrate, observing casts containing multiple cell types is not uncommon. Mixed cellular casts most frequently encountered include RBC and WBC casts in glomerulonephritis and



Figure 6–58 Fatty cast showing adherence of fat droplets to cast matrix (×400).



Figure 6–59 Fatty cast (\times 400).

WBC and RTE cell casts, or WBC and bacterial casts in pyelonephritis.

The presence of mixed elements in a cast may make identification more difficult. Staining or phase microscopy aids in the identification. When mixed casts are present, there should also be homogenous casts of at least one of the cell types, and they will be the primary diagnostic marker. For example, in glomerulonephritis, the predominant casts will be RBC, and in pyelonephritis, the predominant casts will be WBC. Bacteria are often incorporated into WBC casts and provide little additional diagnostic significance. Laboratory protocol should be followed in the reporting of mixed cellular casts.

Granular Casts

Coarsely and finely granular casts are frequently seen in the urinary sediment and may be of pathologic or nonpathologic significance. It is not considered necessary to distinguish between coarsely and finely granular casts.

The origin of the granules in nonpathologic conditions appears to be from the lysosomes excreted by RTE cells during normal metabolism.²⁴ It is not unusual to see hyaline casts containing one or two of these granules. Increased cellular metabolism occurring during periods of strenuous exercise



Figure 6–60 Fatty cast under phase microscopy (×400).

accounts for the transient increase of granular casts that accompany the increased hyaline casts (Figs. 6-61 and 6-62). 15 In disease states, granules may represent disintegration of cellular casts and tubule cells or protein aggregates filtered by the glomerulus (Figs. 6-63 and 6-64). Scanning electron microscope studies have confirmed that granular casts seen in conjunction with WBC casts contain WBC granules of varying



Figure 6-61 Finely granular cast and uric acid crystals (×400).



Figure 6-62 Granular cast formed at a tubular bend (×400).

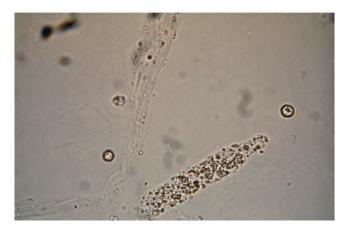


Figure 6–63 Granular disintegrating cellular cast (×400).



Figure 6–64 Coarsely granular cast, squamous epithelial cell, and mucus (×400).

sizes.²⁵ Urinary stasis allowing the casts to remain in the tubules must be present for granules to result from disintegration of cellular casts.

Granular casts occurring as a result of cellular disintegration may contain an occasional recognizable cell. Granular casts are easily visualized under low-power microscopy. However, final identification should be performed using high power to determine the presence of a cast matrix.

Artifacts, such as clumps of small crystals and fecal debris, may occur in shapes resembling casts and must be differentiated. As mentioned previously, columnar RTE cells may also resemble granular casts, and staining for nuclear detail may be required.

When granular casts remain in the tubules for extended periods, the granules further disintegrate, and the cast matrix develops a waxy appearance. The structure becomes more rigid, the ends of the casts may appear jagged or broken, and the diameter becomes broader (Fig. 6-65).

Waxy Casts

Waxy casts are representative of extreme urine stasis, indicating chronic renal failure. They are usually seen in conjunction



Figure 6–65 Granular cast degenerating into waxy cast (×400).

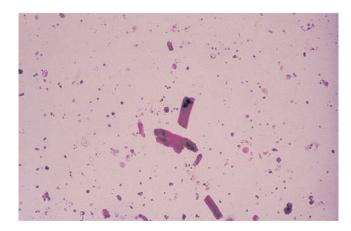


Figure 6–66 KOVA-stained waxy casts (X100).

with other types of casts associated with the condition that has caused the renal failure.

The brittle, highly refractive cast matrix from which these casts derive their name is believed to be caused by degeneration of the hyaline cast matrix and any cellular elements or granules contained in the matrix.^{22,24}

Waxy casts are more easily visualized than hyaline casts because of their higher refractive index. As a result of the brittle consistency of the cast matrix, they often appear fragmented with jagged ends and have notches in their sides (Figs. 6-66 through 6-68). With supravital stains, waxy casts stain a homogenous, dark pink.

Broad Casts

Often referred to as renal failure casts, broad casts like waxy casts represent extreme urine stasis. As a mold of the distal convoluted tubules, the presence of broad casts indicates destruction (widening) of the tubular walls. Also, when the flow of urine to the larger collecting ducts becomes severely compromised, casts form in this area and appear broad.

All types of casts may occur in the broad form. However, considering the accompanying urinary stasis, the most com-



Figure 6-67 KOVA-stained waxy casts (X200).



Figure 6-68 KOVA-stained waxy cast (×400).

monly seen broad casts are granular and waxy (Figs. 6-69 and 6-70). Bile-stained broad, waxy casts are seen as the result of the tubular necrosis caused by viral hepatitis (Fig. 6-71).

Urinary Crystals

Crystals frequently found in the urine are rarely of clinical significance. They may appear as true geometrically formed structures or as amorphous material. The primary reason for the identification of urinary crystals is to detect the presence of the relatively few abnormal types that may represent such disorders as liver disease, inborn errors of metabolism, or renal damage caused by crystallization of *iatrogenic* compounds within the tubules. Crystals are usually reported as rare, few, moderate, or many per hpf. Abnormal crystals may be averaged and reported per lpf.

Crystal Formation

Crystals are formed by the precipitation of urine solutes, including inorganic salts, organic compounds, and medications (iatrogenic compounds). Precipitation is subject to changes in temperature, solute concentration, and pH, which affect solubility.

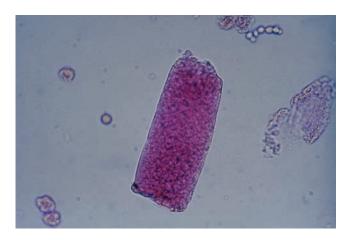


Figure 6-69 KOVA-stained broad waxy cast (×400).

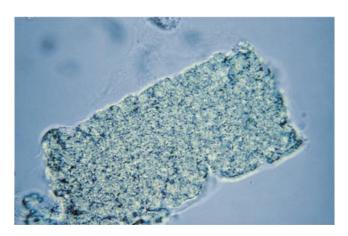


Figure 6-70 Broad granular cast becoming waxy (×400).

Solutes precipitate more readily at low temperatures. Therefore, the majority of crystal formation takes place in specimens that have remained at room temperature or been refrigerated prior to testing. Crystals are extremely abundant in refrigerated specimens and often present problems because they obscure clinically significant sediment constituents.

As the concentration of urinary solutes increases, their ability to remain in solution decreases, resulting in crystal formation. The presence of crystals in freshly voided urine is most frequently associated with concentrated (high specific gravity) specimens.

A valuable aid in the identification of crystals is the pH of the specimen because this determines the type of chemicals precipitated. In general, organic and iatrogenic compounds crystallize more easily in an acidic pH, whereas inorganic salts are less soluble in neutral and alkaline solutions. An exception is calcium oxalate, which precipitates in both acidic and neutral urine.

General Identification Techniques

The most commonly seen crystals have very characteristic shapes and colors; however, variations do occur and can present identification problems, particularly when they resemble

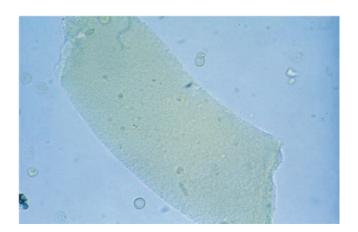


Figure 6–71 Broad bile-stained waxy cast (×400).

Hyaline		Bacterial	
Appearance:	Colorless homogenous matrix	Appearance:	Bacilli bound to protein matrix
Sources of error:	Mucus, fibers, hair, increased lighting	Sources of error:	Granular casts
		Reporting:	Average number per lpf
Reporting:	Average number per lpf		W/DC
Canadataiaalia	Dura train	Complete urinalysis	WBC cast
Complete urinalysis	Protein	correlations:	WBCs
orrelations:	Blood (exercise)		LE NECE
	Color (exercise)		Nitrite
21: 1 : :0			Protein
Clinical significance:	Glomerulonephritis		Bacteria
	Pyelonephritis	Clinian sinai6	Disabasa a haitia
	Chronic renal disease	Clinical significance:	Pyelonephritis
	Congestive heart failure Stress and exercise	Epithelial Cell	
RBC		Appearance:	RTE cells attached to protein matrix
Appearance:	Orange-red color, cast matrix		
	containing RBCs	Sources of error:	WBC cast
Sources of error:	RBC clumps	Reporting:	Average number per lpf
	·	· -	
Reporting:	Average number per lpf	Complete urinalysis	Protein
		correlations:	RTE cells
Complete urinalysis	RBCs		
correlations:	Blood	Clinical significance:	Renal tubular damage
	Protein		
		Granular	
Clinical significance:	Glomerulonephritis		
	Strenuous exercise	Appearance:	Coarse and fine granules in a cast matrix
NBC			
		Sources of error:	Clumps of small crystals
Appearance:	Cast matrix containing		Columnar RTE cells
	WBCs		
		Reporting:	Average number per lpf
ources of error:	WBC clumps		
	·	Complete urinalysis	Protein
Reporting:	Average number per lpf	correlations:	Cellular casts
			RBCs
Complete urinalysis	WBCs		WBCs
orrelations:	Protein		
	LE	Clinical significance:	Glomerulonephritis
Clinical significance:	Pyelonephritis		Pyelonephritis Stress and exercise
linical cignificanco	Pyelonephritis		Stress and eversise

Summary of Urine Casts (continued) Waxy Reporting: Average number per lpf Complete urinalysis Protein Highly refractile cast with jagged Appearance: correlations: Free fat droplets ends and notches Oval fat bodies Sources of error: Fibers and fecal material Nephrotic syndrome Clinical significance: Toxic tubular necrosis Reporting: Average number per lpf Diabetes mellitus Crush injuries Complete urinalysis Protein correlations: Cellular casts **Broad** Granular casts **WBCs** Appearance: Wider than normal cast matrix **RBCs** Sources of error: Fecal material, fibers Clinical significance: Stasis of urine flow Reporting: Average number per lpf Chronic renal failure Complete urinalysis Protein **Fatty** correlations: **WBCs RBCs** Fat droplets and oval fat Appearance: Granular casts bodies attached to protein Waxy casts matrix Clinical significance: Extreme urine stasis Sources of error: Fecal debris Renal failure

abnormal crystals. As discussed previously, the first consideration when identifying crystals is the urine pH. In fact, crystals are routinely classified not only as normal and abnormal, but also as to their appearance in acidic or alkaline urine. All abnormal crystals are found in acidic urine.

Additional aids in crystal identification include the use of polarized microscopy and solubility characteristics of the crystals. The geometric shape of a crystal determines its birefringence and, therefore, its ability to polarize light. Although the size of a particular crystal may vary (slower crystallization produces larger crystals), the basic structure remains the same. Therefore, polarization characteristics for a particular crystal are constant for identification purposes.

Just as changes in temperature and pH contribute to crystal formation, reversal of these changes can cause crystals to dissolve. These solubility characteristics can be used to aid in identification. Amorphous urates that frequently form in refrigerated specimens and obscure sediments may dissolve if the specimen is warmed. Amorphous phosphates require acetic acid to dissolve, and this is not practical, as formed elements, such as RBCs, will also be destroyed. When solubility characteristics are needed for identification, the sediment should be aliquoted to prevent destruction of other elements. In Table 6–6, solubility characteristics for the most commonly encountered crystals are provided.

Normal Crystals Seen in Acidic Urine

The most common crystals seen in acidic urine are urates, consisting of amorphous urates, uric acid, acid urates, and sodium urates. Microscopically, most urate crystals appear yellow to reddish brown and are the only normal crystals found in acidic urine that appear colored.

Amorphous urates appear microscopically as yellow-brown granules (Fig. 6-72). They may occur in clumps resembling granular casts. Amorphous urates are frequently encountered in specimens that have been refrigerated and produce a very characteristic pink sediment. Accumulation of the pigment, uroerythrin, on the surface of the granules is the cause of the pink color. Amorphous urates are found in acidic urine with a pH greater than 5.5, whereas uric acid crystals can appear when the pH is lower.

Uric acid crystals are seen in a variety of shapes, including rhombic, four-sided flat plates (whetstones), wedges, and rosettes. They usually appear yellow-brown, but may be colorless and have a six-sided shape, similar to cystine crystals (Figs. 6-73 and 6-74). Uric acid crystals are highly birefringent under polarized light, which aids in distinguishing them from cystine crystals (Figs. 6-75 and 6-76). Increased amounts of uric acid crystals, particularly in fresh urine, are associated with increased levels of purines and nucleic acids

Crystal	pН	Color	Solubility	Appearance
Uric acid	Acid	Yellow-brown	Alkali soluble	
Amorphous urates	Acid	Brick dust or yellow brown	Alkali and heat	
Calcium oxalate	Acid/neutral (alkaline)	Colorless (envelopes, oval, dumbbell)	Dilute HCl oval	
Amorphous phosphates	Alkaline Neutral	White-colorless	Dilute acetic acid	
Calcium phosphate	Alkaline Neutral	Colorless	Dilute acetic acid	
Triple phosphate	Alkaline	Colorless ("coffin lids")	Dilute acetic acid	
Ammonium biurate	Alkaline	Yellow-brown ("thorny apples")	Acetic acid with heat	
Calcium carbonate	Alkaline	Colorless (dumbbells)	Gas from acetic acid	Can 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2

and are seen in patients with leukemia who are receiving chemotherapy, in patients with Lesch-Nyhan syndrome (see Chapter 9), and, sometimes, in patients with gout.

Acid urates and sodium urates are rarely encountered and, like amorphous urates, are seen in less acidic urine. They are frequently seen in conjunction with amorphous urates and have little clinical significance. Acid urates appear as larger granules and may have spicules similar to the ammonium biurate crystals seen in alkaline urine. Sodium urate crystals are needle-shaped and are seen in synovial fluid during episodes of gout, but do appear in the urine.

Calcium oxalate crystals are frequently seen in acidic urine, but they can be found in neutral urine and even rarely in alkaline urine. The most common form of calcium oxalate crystals is the dihydrate that is easily recognized as a colorless, octahedral envelope or as two pyramids joined at their bases (Figs. 6-77 and 6-78). Less characteristic and less frequently seen is the monohydrate form (Fig. 6-79). Monohydrate calcium oxalate crystals are oval or dumbbell shaped. Both the dihydrate and monohydrate forms are birefringent under polarized light. This may be helpful to distinguish the monohydrate form from nonpolarizing RBCs. Calcium oxalate crys-

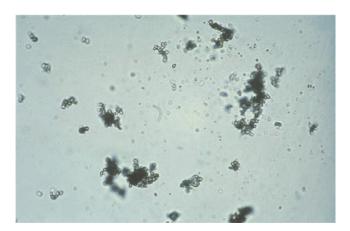


Figure 6–72 Amorphous urates (×400).

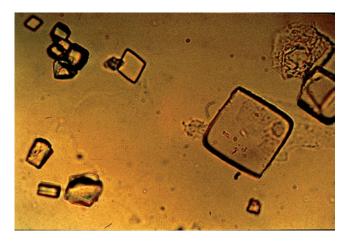


Figure 6–73 Uric acid crystals (×400).

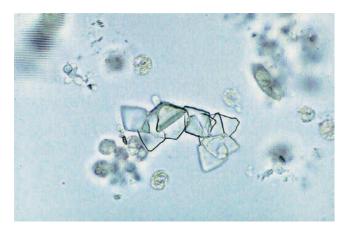


Figure 6–74 Clump of uric acid crystals (×400). Notice the whetstone, not hexagonal, shape that differentiates uric acid from cystine crystals.

tals are sometimes seen in clumps attached to mucous strands and may resemble casts.

The finding of clumps of calcium oxalate crystals in fresh urine may be related to the formation of renal calculi, because the majority of renal calculi are composed of calcium



Figure 6–75 Uric acid crystals under polarized light (×100).



Figure 6–76 Uric acid crystals under polarized light (×400).



Figure 6–77 Classic dihydrate calcium oxalate crystals (×400).

oxalate. They are also associated with foods high in oxalic acid, such as tomatoes and asparagus, and ascorbic acid, because oxalic acid is an end product of ascorbic acid metabolism. The primary pathologic significance of calcium oxalate crystals is the very noticeable presence of the mono-

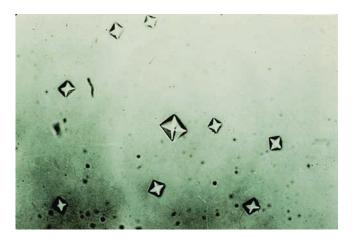


Figure 6–78 Classic dehydrate calcium oxalate crystals under phase microscopy (×400).

hydrate form in cases of ethylene glycol (antifreeze) poisoning. Massive amounts of crystals are frequently produced in these cases.

Normal Crystals Seen in Alkaline Urine

Phosphates represent the majority of the crystals seen in alkaline urine and include amorphous phosphate, triple phosphate, and calcium phosphate. Other normal crystals associated with alkaline urine are calcium carbonate and ammonium biurate. Amorphous phosphates are granular in appearance, similar to amorphous urates (Fig. 6-80). When present in large quantities following specimen refrigeration, they cause a white precipitate that does not dissolve on warming. They can be differentiated from amorphous urates by the color of the sediment and the urine pH.

Triple phosphate (ammonium magnesium phosphate) crystals are commonly seen in alkaline urine. In their routine form, they are easily identified by their prism shape that frequently resembles a "coffin lid" (Figs. 6-81 and 6-82). As they disintegrate, the crystals may develop a feathery appearance. Triple phosphate crystals are birefringent under polarized



Figure 6–79 Monohydrate calcium oxalate crystals (X400).

light. They have no clinical significance; however, they are often seen in highly alkaline urine associated with the presence of urea-splitting bacteria (Fig. 6-83).

Calcium phosphate crystals are not frequently encountered. They may appear as colorless, flat rectangular plates or thin prisms often in rosette formations. The rosette forms may

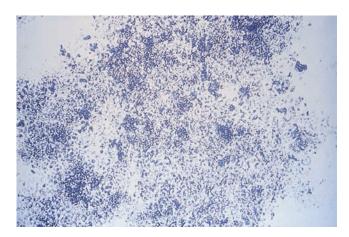


Figure 6–80 Amorphous phosphates (×400).

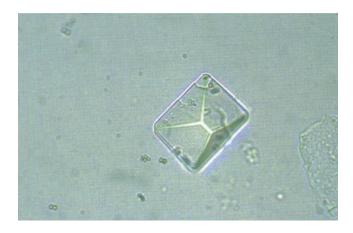


Figure 6–81 Triple phosphate crystal (×400).

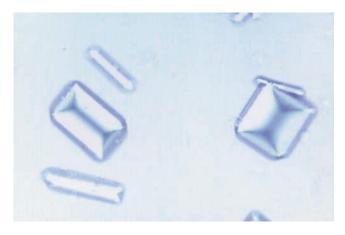


Figure 6–82 "Coffin lid" and other forms of triple phosphate crystals (X400).

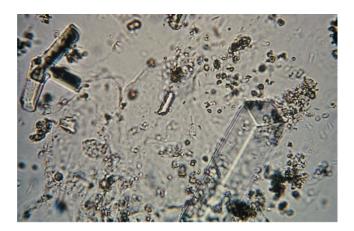


Figure 6–83 Triple phosphate crystals and amorphous phosphates (×400).

be confused with sulfonamide crystals when the urine pH is in the neutral range. Calcium phosphate crystals dissolve in dilute acetic acid and sulfonamides do not. They have no clinical significance, though calcium phosphate is a common constituent of renal calculi.

Calcium carbonate crystals are small and colorless, with dumbbell or spherical shapes (Fig. 6-84). They may occur in clumps that resemble amorphous material, but they can be distinguished by the formation of gas after the addition of acetic acid. They are also birefringent, which differentiates them from bacteria. Calcium carbonate crystals have no clinical significance.

Ammonium biurate crystals exhibit the characteristic yellow-brown color of the urate crystals seen in acidic urine. They are frequently described as "thorny apples" because of their appearance as spicule-covered spheres (Fig. 6-85). Except for their occurrence in alkaline urine, ammonium biurate crystals resemble other urates in that they dissolve at 60°C and convert to uric acid crystals when glacial acetic acid is added. Ammonium biurate crystals are almost always encountered in old specimens and may be associated with the presence of the ammonia produced by urea-splitting bacteria.



Figure 6–84 Calcium carbonate crystals (×400).

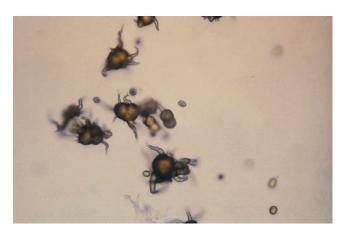


Figure 6–85 Ammonium biurate crystals (X400). (Courtesy of Kenneth L. McCoy, MD.)

Abnormal Urine Crystals

Abnormal urine crystals are found in acidic urine or rarely in neutral urine. Most abnormal crystals have very characteristic shapes. However, their identity should be confirmed by chemical tests (Table 6–7) or by patient information (medications). Iatrogenic crystals can be caused by a variety of compounds, particularly when they are administered in high concentrations. They may be of clinical significance when they precipitate in the renal tubules. The most commonly encountered iatrogenic crystals are discussed in this section.

Cystine Crystals

Cystine crystals are found in the urine of persons who inherit a metabolic disorder that prevents reabsorption of cystine by the renal tubules (cystinuria). Persons with cystinuria have a tendency to form renal calculi, particularly at an early age.

Cystine crystals appear as colorless, hexagonal plates and may be thick or thin (Figs. 6-86 and 6-87). Disintegrating forms may be seen in the presence of ammonia. They may be difficult to differentiate from colorless uric acid crystals. Uric acid crystals are very birefringent under polarized microscopy, whereas only thick cystine crystals have polarizing capability. Positive confirmation of cystine crystals is made using the cyanide-nitroprusside test (see Chapter. 9).

Cholesterol Crystals

Cholesterol crystals are rarely seen unless specimens have been refrigerated, because the lipids remain in droplet form. However, when observed, they have a most characteristic appearance, resembling a rectangular plate with a notch in one or more corners (Fig. 6-88). They are associated with disorders producing lipiduria, such as the nephrotic syndrome, and are seen in conjunction with fatty casts and oval fat bodies. Cholesterol crystals are highly birefringent with polarized light (Fig. 6-89).

Radiographic Dye Crystals

Crystals of radiographic contrast media have a very similar appearance to cholesterol crystals and also are highly birefringent.

Table 6–7 M	ajor Chara	cteristics of Abno	ormal Urinary Cryst	als
Crystal	рН	Color	Solubility	Appearance
Cystine	Acid	Colorless	Ammonia, dilute HCl	
Cholesterol	Acid	Colorless (notched plates)	Chloroform	a Per
Leucine	Acid/neutral	Yellow	Hot alkali or alcohol	
Tyrosine	Acid/neutral	Colorless–yellow	Alkali or heat	
Bilirubin	Acid	Yellow	Acetic acid, HCl, NaOH, ether, chloroform	***
Sulfonamides	Acid/neutral	Varied	Acetone	
Radiographic dye	Acid	Colorless	10% NaOH	000
Ampicillin	Acid/neutral	Colorless	Refrigeration forms bundles	

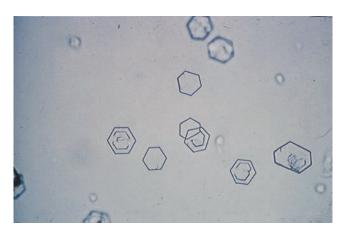


Figure 6–86 Cystine crystals (×400).

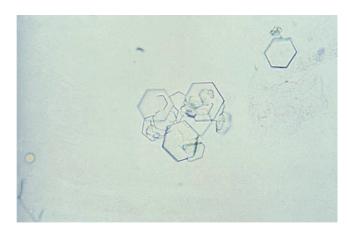


Figure 6–87 Clump of cystine crystals (\times 400). Notice the hexagonal shape still visible.

Figure 6–88 Cholesterol crystals. Notice the notched corners (×400).

Differentiation is best made by comparison of the other urinalysis results and the patient history. As mentioned previously, cholesterol crystals should be accompanied by other lipid elements and heavy proteinuria. Likewise, the specific gravity of a specimen containing radiographic contrast media is markedly elevated when measured by refractometer.

Crystals Associated With Liver Disorders

In the presence of severe liver disorders, three rarely seen crystals may be found in the urine sediment. They are crystals of tyrosine, leucine, and bilirubin.

Tyrosine crystals appear as fine colorless to yellow needles that frequently form clumps or rosettes (Figs. 6-90 and 6-91). They are usually seen in conjunction with leucine crystals in specimens with positive chemical test results for bilirubin. Tyrosine crystals may also be encountered in inherited disorders of amino-acid metabolism (see Chapter 9).

Leucine crystals are yellow-brown spheres that demonstrate concentric circles and radial striations (Fig. 6-92). They are seen less frequently than tyrosine crystals and, when present, should be accompanied by tyrosine crystals.

Bilirubin crystals are present in hepatic disorders producing large amounts of bilirubin in the urine. They appear as clumped needles or granules with the characteristic yellow

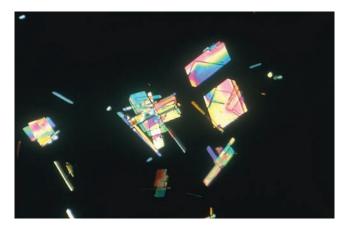


Figure 6–89 Cholesterol crystals under polarized light (×400).

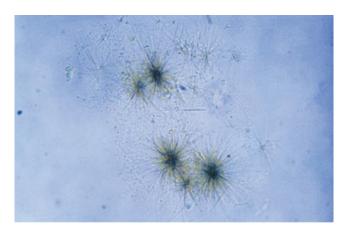


Figure 6–90 Tyrosine crystals in fine needle clumps (×400).



Figure 6–91 Tyrosine crystals in rosette forms (×400).

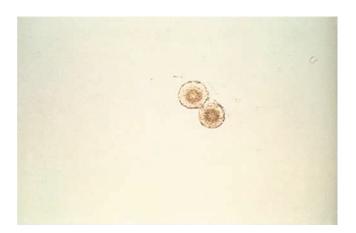


Figure 6–92 Leucine crystals (×400).

color of bilirubin (Fig. 6-93). A positive chemical test result for bilirubin would be expected. In disorders that produce renal tubular damage, such as viral hepatitis, bilirubin crystals may be found incorporated into the matrix of casts.

Sulfonamide Crystals

Prior to the development of more soluble sulfonamides, the finding of these crystals in the urine of patients being treated

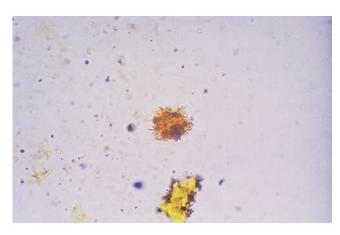


Figure 6–93 Bilirubin crystals. Notice the classic bright yellow color (×400).

for UTIs was common. Inadequate patient hydration was and still is the primary cause of sulfonamide crystallization. The appearance of sulfonamide crystals in fresh urine can suggest the possibility of tubular damage if crystals are forming in the nephron.

A variety of sulfonamide medications are currently on the market; therefore, one can expect to encounter a variety of crystal shapes and colors. Shapes most frequently encountered include needles, rhombics, whetstones, sheaves of wheat, and rosettes with colors ranging from colorless to yellow-brown (Figs. 6-94 and 6-95). A check of the patient's medication history aids in the identification confirmation. If necessary, a diazo reaction can be performed for further confirmation.

Ampicillin Crystals

Precipitation of antibiotics is not frequently encountered except for the rare observation of ampicillin crystals following massive doses of this penicillin compound without adequate hydration. Ampicillin crystals appear as colorless needles that tend to form bundles following refrigeration (Figs. 6-96 and 6-97). Knowledge of the patient's history can aid in the identification.

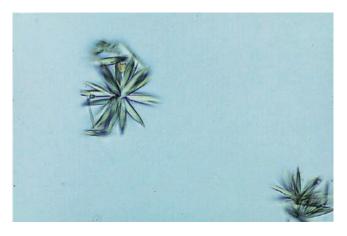


Figure 6-94 Sulfa crystals in rosette form (×400).



Figure 6–95 Sulfa crystals, WBCs, and bacteria seen in UTI $(\times 400)$.



Figure 6–96 Ampicillin crystals, nonrefrigerated (×400).

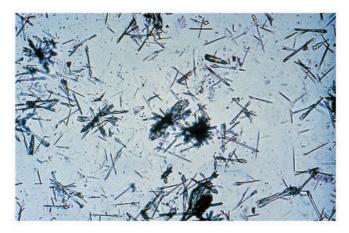


Figure 6–97 Ampicillin crystals following refrigeration (×400).

Urinary Sediment Artifacts

Contaminants of all types can be found in urine, particularly in specimens collected under improper conditions or in dirty containers. The most frequently encountered artifacts include starch, oil droplets, air bubbles, pollen grains, fibers, and fecal

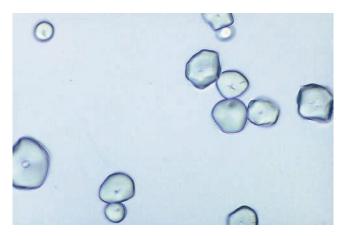


Figure 6–98 Starch granules. Notice the dimpled center (×400).

contamination. Because artifacts frequently resemble pathologic elements such as RBCs and casts, artifacts can present a major problem to students. They are often very highly refractile or occur in a different microscopic plane than the true sediment constituents. The reporting of artifacts is not necessary.

Starch granule contamination may occur when cornstarch is the powder used in powdered gloves. The granules are highly refractile spheres, usually with a dimpled center (Fig. 6-98). They resemble fat droplets when polarized, producing a Maltese cross formation. Starch granules may also occasionally be confused with RBCs. Differentiation between starch and pathologic elements can be made by considering other urinalysis results, including chemical tests for blood or protein and the presence of oval fat bodies or fatty casts.

Oil droplets and air bubbles also are highly refractile and may resemble RBCs to inexperienced laboratory personnel. Oil droplets may result from contamination by immersion oil or lotions and creams (Fig. 6-99). Air bubbles occur when the specimen is placed under a cover slip. The presence of these artifacts should be considered in the context of the other urinalysis results.

Pollen grains are seasonal contaminants that appear as spheres with a cell wall and occasional concentric circles (Fig.

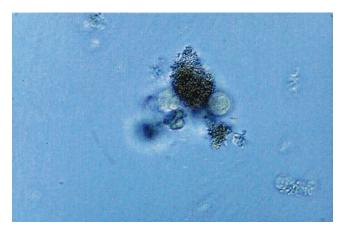


Figure 6–99 Fecal material and oil artifacts (×400).

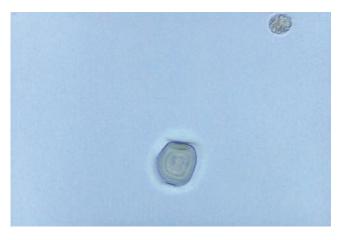


Figure 6–100 Pollen grain. Notice the concentric circles (×400).

6-100). Like many artifacts, their large size may cause them to be out of focus with true sediment constituents.

Hair and fibers from clothing and diapers may initially be mistaken for casts (Figs. 6-101 and 6-102), though they are usually much longer and more refractile. Examination under polarized light can frequently differentiate between



Figure 6–101 Fiber resembling a cast (\times 400).

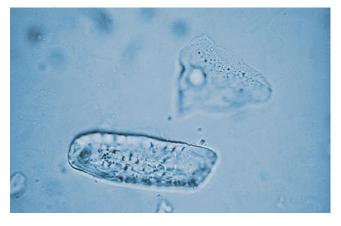


Figure 6–102 Diaper fiber resembling a cast. Notice the refractility (×400).

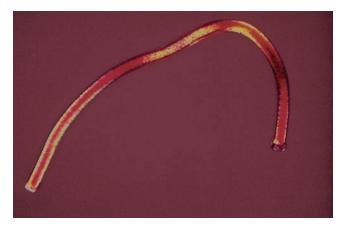


Figure 6–103 Fiber under polarized light (×100).



Figure 6–104 Vegetable fiber resembling waxy cast (×400).

fibers and casts (Fig. 6-103). Fibers often polarize, whereas, casts, other than fatty casts, do not.

Improperly collected specimens or rarely the presence of a fistula between the intestinal and urinary tracts may produce fecal specimen contamination. Fecal artifacts may appear as plant and meat fibers or as brown amorphous material in a variety of sizes and shapes (Fig. 6-104).

References

- 1. Mynahan, C: Evaluation of macroscopic urinalysis as a screening procedure. Lab Med 15(3):176-179, 1984.
- Tetrault, GA: Automated reagent strip urinalysis: Utility in reducing work load of urine microscopy and culture. Lab Med 25:162-167, 1994.
- 3. Clinical and Laboratory Standards Institute (CLSI), formerly NCCLS: Urinalysis and Collection, Transportation, and Preserva-

- tion of Urine Specimens; Approved Guideline GP16-A2, Second Edition, Wayne, Pa., 2001.
- 4. Addis, T: The number of formed elements in the urinary sediment of normal individuals. J Clin Invest 2(5):409-415, 1926.
- Schumann, GB, and Tebbs, RD: Comparison of slides used for standardized routine microscopic urinalysis. J Med Technol 3(1):54-58, 1986.
- Sternheimer, R, and Malbin, R: Clinical recognition of pyelonephritis with a new stain for urinary sediments. Am J Med 11:312-313, 1951.
- 7. Microscope Techniques—Phase Contrast: http://www.micro.magnet.fsuj.edu/primer/techniques/phase.html
- 8. Polarizing and Interference Contrast Microscopy: http://www.rrz.uni-hamburg.de/biologic/b
- Olympus Microscopy Resource Center: Specialized Microscopy Techniques: Fluorescence. http://www.olympusmicro.com/ primer/techniques/fluorescence/fluorhome.html. Accessed December 19, 2006.
- 10. Simpson, LO: Effects of normal and abnormal urine on red cell shape. Nephron 60(3):383-384, 1992.
- 11. Stapleton, FB: Morphology of urinary red blood cells: A simple guide in localizing the site of hematuria. Pediatr Clin North Am 34(3):561-569, 1987.
- 12. Fassett, EG, et al: Urinary red cell morphology during exercise. Am J Clin Pathol 285(6353):1455-1457, 1982.
- Kohler, H, Wandel, E, and Brunch, B: Acanthocyturia: A characteristic marker for glomerular bleeding. Int Soc Nephrol 40:115-120, 1991.
- 14. Tomita, M, et al: A new morphological classification of urinary erythrocytes for differential diagnosis of hematuria. Clin Nephrol 37(2):84-89, 1992.
- 15. Haber, MH, Lindner, LE, and Ciofalo, LN: Urinary casts after stress. Lab Med 10(6):351-355, 1979.
- Corwin, HL, Bray, RA, and Haber, MH: The detection and interpretation of urinary eosinophils. Arch Pathol Lab Med 113:1256-1258, 1989.
- 17. Schumann, GB: Utility of urinary cytology in renal diseases. Semin Nephrol 5(34) Sept, 1985.
- Graber, M, et al: Bubble cells: Renal tubular cells in the urinary sediment with characteristics of viability. J Am Soc Nephrol 1(7):999-1004, 1991.
- 19. Baer, DM: Tips from clinical experts: Reporting of spermatozoa in microscopic urine exams. MLO 12:12, 1997.
- 20. Kumar, S, and Muchmore, A: Tamm-Horsfall protein— Uromodulin, 1950–1990. Kidney Int 37:1395-1399, 1990.
- 21. Haber, MH: Urinary Sediment: A Textbook Atlas. American Society of Clinical Pathologists, Chicago, 1981.
- Lindner, LE, and Haber, MH: Hyaline casts in the urine: Mechanism of formation and morphological transformations. Am J Clin Pathol 80(3):347-352, 1983.
- 23. Lindner, LE, Jones, RN, and Haber, MH: A specific cast in acute pyelonephritis. Am J Clin Pathol 73(6):809-811, 1980.
- 24. Haber, MH, and Lindner, LE: The surface ultrastructure of urinary casts. Am J Clin Pathol 68(5):547-552, 1977.
- Linder, LE, Vacca, D, and Haber, MF: Identification and composition of types of granular urinary cast. Am J Pathol 80(3):353-358, 1983.

QUESTIONS STUDY

- 1. Macroscopic screening of urine specimens is used to:
 - A. Provide results as soon as possible
 - B. Predict the type of urinary casts present
 - C. Increase cost-effectiveness of urinalysis
 - D. Decrease the need for polarized microscopy
- **2.** Variations in the microscopic analysis of urine include all of the following *except*:
 - A. Preparation of the urine sediment
 - B. Amount of sediment analyzed
 - C. Method of reporting
 - D. Identification of formed elements
- **3.** All of the following can cause false-negative microscopic results *except*:
 - A. Braking the centrifuge
 - B. Failing to mix the specimen
 - C. Dilute alkaline urine
 - D. Using midstream clean-catch specimens
- **4.** The two factors that determine relative centrifugal force are:
 - A. Radius of rotor head and rpm
 - B. Radius of rotor head and time of centrifugation
 - C. Diameter of rotor head and rpm
 - D. RPM and time of centrifugation
- **5.** When using the glass slide and coverslip method, which of the following might be missed if the coverslip is overflowed?
 - A. Casts
 - B. RBCs
 - C. WBCs
 - D. Bacteria
- **6.** Initial screening of the urine sediment is performed using an objective power of:
 - A. 4×
 - B. 10×
 - C. 40×
 - D. 100×
- 7. Which of the following should be used to reduce light intensity in bright-field microscopy?
 - A. Centering screws
 - B. Aperture diaphragm
 - C. Rheostat
 - D. Condenser aperture diaphrgam
- **8.** Which of the following are reported as number per LPF?
 - A. RBCs
 - B. WBCs
 - C. Crystals
 - D. Casts

- **9.** The Sternheimer-Malbin stain is added to urine sediments to do all of the following *except*:
 - A. Increase visibility of sediment constituents
 - B. Change the constituents refractive index
 - C. Decrease precipitation of crystals
 - D. Delineate constituent structures
- 10. Nuclear detail can be enhanced by:
 - A. Prussian blue
 - B. Toluidine blue
 - C. Acetic acid
 - D. Both B and C
- 11. Which of the following lipids is/are stained by Sudan III?
 - A. Cholesterol
 - B. Neutral fats
 - C. Triglycerides
 - D. Both B and C
- **12.** Which of the following lipids is/are capable of polarizing light?
 - A. Cholesterol
 - B. Neutral fats
 - C. Triglycerides
 - D. Both A and B
- **13**. The purpose of the Hansel stain is to identify:
 - A. Neutrophils
 - B. Renal tubular cells
 - C. Eosinophils
 - D. Monocytes
- 14. Crenated RBCs are seen in urine that is:
 - A. Hyposthenuric
 - B. Hypersthenuric
 - C. Highly acidic
 - D. Highly alkaline
- **15**. Differentiation among RBCs, yeast, and oil droplets may be accomplished by all of the following *except*:
 - A. Observation of budding in yeast cells
 - B. Increased refractility of oil droplets
 - C. Lysis of yeast cells by acetic acid
 - D. Lysis of RBCs by acetic acid
- **16**. The finding of dysmorphic RBCs is indicative of:
 - A. Glomerular bleeding
 - B. Renal calculi
 - C. Traumatic injury
 - D. Coagulation disorders
- **17**. Leukocytes that stain pale blue with Sternheimer-Malbin stain and exhibit brownian movement are:
 - A. Indicative of pyelonephritis
 - B. Basophils
 - C. Mononuclear leukocytes
 - D. Glitter cells

- **18.** Mononuclear leukocytes are sometimes mistaken for:
 - A. Yeast cells
 - B. Squamous epithelial cells
 - C. Pollen grains
 - D. Renal tubular cells
- **19**. When pyuria is detected in a sediment, the slide should be carefully checked for the presence of:
 - A. RBCs
 - B. Bacteria
 - C. Hyaline casts
 - D. Mucus
- **20**. Transitional epithelial cells are sloughed from the:
 - A. Collecting duct
 - B. Vagina
 - C. Bladder
 - D. Proximal convoluted tubule
- **21**. The largest cells in the urine sediment are:
 - A. Squamous epithlial cells
 - B. Urothelial epithelial cells
 - C. Cuboidal epithelial cells
 - D. Columnar epithelial cells
- **22**. A clinically significant squamous epithelial cell is the:
 - A. Cuboidal cell
 - B. Clue cell
 - C. Caudate cell
 - D. Columnar cell
- **23.** Forms of transitional epithelial cells include all of the following *except*:
 - A. Spherical
 - B. Caudate
 - C. Convoluted
 - D. Polyhedral
- 24. Increased transitional cells are indicative of:
 - A. Catheterization
 - B. Malignancy
 - C. Pyelonephritis
 - D. Both A and B
- **25.** A primary characteristic used to identify renal tubular epithelial cells is:
 - A. Elongated structure
 - B. Centrally located nucleus
 - C. Spherical appearance
 - D. Eccentrically located nucleus
- **26.** Following an episode of hemoglobinuria, RTE cells may contain:
 - A. Bilirubin
 - B. Hemosiderin granules
 - C. Porphobilinogen
 - D. Myoglobin

- **27**. The predecessor of the oval fat body is the:
 - A. Histiocyte
 - B. Urothelial cell
 - C. Monocyte
 - D. Renal tubular cell
- **28.** A structure believed to be an oval fat body produced a Maltese cross formation under polarized light but does not stain with Sudan III. The structure:
 - A. Contains cholesterol
 - B. Is not an oval fat body
 - C. Contains neutral fats
 - D. Is contaminated with immersion oil
- **29**. The finding of yeast cells in the urine is commonly associated with:
 - A. Cystitis
 - B. Diabetes mellitus
 - C. Pyelonephritis
 - D. Liver disorders
- **30**. The primary component of urinary mucus is:
 - A. Bence Jones protein
 - B. Microalbumin
 - C. Tamm-horsfall protein
 - D. Orthostatic protein
- **31**. The majority of casts are formed in the:
 - A. Proximal convoluted tubules
 - B. Ascending loop of Henle
 - C. Distal convoluted tubules
 - D. Collecting ducts
- **32.** Cylindroiduria refers to the presence of:
 - A. Cylindrical renal tubular cells
 - B. Mucus resembling casts
 - C. Hyaline and waxy casts
 - D. All types of casts
- **33.** A person submitting a urine specimen following a strenuous exercise routine can normally have all of the following in the sediment *except*:
 - A. Hyaline casts
 - B. Granular casts
 - C. RBC casts
 - D. WBC casts
- **34.** Prior to identifying an RBC cast, all of the following should be observed *except*:
 - A. Free-floating RBCs
 - B. Intact RBCs in the cast
 - C. Presence of a cast matrix
 - D. A positive reagent strip blood reaction
- **35.** WBC casts are primarily associated with:
 - A. Pyelonephritis
 - B. Cystitis
 - C. Glomerulonephritis
 - D. Viral infections

Continued
36. The shape of the RTE cell associated with renal tubular epithelial casts is primarily:A. ElongatedB. CuboidalC. RoundD. Columnar
37. When observing RTE casts, the cells are primarily:A. Embedded in a clear matrixB. Embedded in a granular matrixC. Attached to the surface of a matrixD. Stained by components of the urine filtrate
38. The presence of fatty casts is associated with: A. Nephrotic syndrome B. Crush injuries C. Diabetes mellitus D. All of the above
39. Nonpathogenic granular casts contain:A. Cellular lysosomesB. Degenerated cellsC. Protein aggregatesD. Gram-positive cocci
40. All of the following are true about waxy casts <i>except</i> they:A. Represent extreme urine stasisB. May have a brittle consistencyC. Require staining to be visualizedD. Contain degenerated granules
41. The observation of broad casts represents: A. Destruction of tubular walls B. Dehydraton and high fever C. Formation in the collecting ducts D. Both A and C
42. All of the following contribute to the formation of urinary crystals <i>except</i>:A. Protein concentrationB. phC. Solute concentrationD. Temperature
 43. The most valuable initial aid for the identification of crystals in a urine specimen is: A. ph B. Solubility C. Staining D. Polarized microscopy
44. Crystals associated with severe liver disease include all of the following <i>except</i>:A. BilirubinB. LeucineC. CystineD. Tyrosine

45.	All of the following crystals routinely polarize <i>except</i> : A. Uric acid B. Cholesterol C. Radiographic dye D. Cystine				
46.	Differentiation between casts and fibers can usually be made using: A. Solubility characteristics B. Patient history C. Polarized light D. Fluorescent light				
47.	Match the followin their description/idAmorphous uUric acidCalcium oxala monhydrateCalcium oxala dihydrate	entify rates ite	 Envelopes Thin needles Yellow-brown, whetstone 		
48.	with their descripti Triple phosph	on/ide ate hosph phate iurate	stals seen in alkaline urine entifying characteristics: 1. Yellow granules nate 2. Thin prisms 3. "Coffin lids" 4. Dumbbell shape 5. White precipitate 6. Thorny apple		
49.	description/identifyCystineTyrosineCholesterolLeucineAmpicillin	ving ch 1. Bu 2. Hig 3. Bri 4. He 5. Fla 6. Co stri 7. No 8. Fir	cormal crystals with their haracteristics: andles following refrigeration ighly alkaline pH right yellow clumps exagonal plates at plates, high specific gravity concentric circles, radial riations otched corners ne needles seen in liver sease		
50.	Match the followin descriptions:Bright-fieldPhasePolarizedDark-fieldFluorescentInterference contrast	 Incoobj Obea Low ma This For obj Deterror 	direct light is reflected off the eject objects split light into two ams ow refractive index objects ay be overlooked aree-dimensional images orms halo of light around eject of the effects electrons emitted om objects		
			etects specific wavelengths of ht emitted from objects		

Case Studies and Clinical Situations

1. An 85-year-old women with diabetes and a broken hip has been confined to bed for the past 3 months. Results of an ancillary blood glucose test are 250 mg/dL, and her physician orders additional blood tests and a routine urinalysis. The urinalysis report is as follows:

COLOR: Pale yellow
CLARITY: Hazy
SP. GRAVITY: 1.020
BILIRUBIN: Negative
PH: 5.5
UROBILINOGEN: Normal
PROTEIN: Trace
GLUCOSE: 100 mg/dL

KETONES: Negative
BLOOD: Moderate
BILIRUBIN: Negative
UROBILINOGEN: Normal
NITRITE: Negative
LEUKOCYTES: 2+

Microscopic: 20 to 25 WBCs/hpf

Many yeast cells and hyphae

- a. Why are yeast infections common in patients with diabetes mellitus?
- b. With a blood glucose level of 250 mg/dL, should glucose be present in the urine? Why or why not?
- c. Is there a discrepancy between the negative nitrite and the positive leukocyte esterase results? Explain your answer.
- d. What is the major discrepancy between the chemical and microscopic results?
- e. Considering the patient's history, what is the most probable cause for the discrepancy?
- 2. A medical technology student training in a newly renovated STAT laboratory is having difficulty performing a microscopic urinalysis. Reagent strip testing indicates the presence of moderate blood and leukocytes, but the student is also observing some large unusual objects resembling crystals and possible casts. The student is also having difficulty keeping all of the constituents in focus at the same time.
 - a. Why is the student having difficulty focusing?
 - b. What is a possible cause of the unusual microscopic constituents?
 - c. Should the student be concerned about the unusual microscopic constituents? Explain your
 - d. What microscopy technique could be used to aid in differentiating a cast and an artifact?
- 3. A prisoner sentenced to 10 years for selling illegal drugs develops jaundice, lethargy, and hepatomegaly. A test for hepatitis B surface antigen is positive, and the patient is placed in the prison infirmary. When his condition appears to worsen and a low urinary output is observed, the patient is transferred to a local hospital. Additional testing detects a superinfection with delta hepatitis virus and decreased renal concentrating ability. Urinalysis results are as follows:

COLOR: Amber KETONES: Negative CLARITY: Hazy BLOOD: Negative

ph: 7.0 urobilinogen: 4.0 EU
protein: 2+ nitrite: Negative
glucose: Negative
Microscopic:

2 to 4 WBCs/hpf 1 to 2 hyaline casts/lpf 1 to 3 RBCs/hpf 1 to 2 granular casts/lpf

2 to 4 bile-stained RTE cells/hpf

0 to 1 RTE casts/lpf

BILIRUBIN: Large

0 to 1 bile-stained waxy casts/lpf

SP. GRAVITY: 1.011

- a. Based on the urinalysis results, in what area of the nephron is damage occurring?
- b. Is this consistent with the patient's primary diagnosis? Explain your answer.
- c. What is causing the RTE cells to be bile stained?
- d. Why is the urobilinogen level elevated?
- e. State a disorder in which the urobilinogen level would be elevated, but the bilirubin result would be negative.
- 4. A 30-year-old woman being treated for a UTI brings a urine specimen to the Employee Health Clinic at 4:00 p.m. The nurse on duty tells her that the specimen will be refrigerated and tested by the technologist the next morning. The technologist has difficulty interpreting the color of the reagent strip tests and reports only the following results:

COLOR: Amber CLARITY: Slightly cloudy Microscopic: 3 to 5 RBCs/hpf 8 to 10 WBCs/hpf

Moderate bacteria

Moderate colorless crystals appearing in bundles

- a. What could have caused the technologist to have difficulty interpreting the reagent strip results?
- b. Could this specimen produce a yellow foam when shaken?
- c. How could it be checked for the presence of bilirubin? Would this really be necessary?
- d. What could the technologist do to aid in the identification of the crystals?
- e. What is the probable identification of the colorless crystals?
- 5. A 2-year-old left unattended in the garage for 5 minutes is suspected of ingesting antifreeze (ethylene glycol). The urinalysis has a pH of 6.0 and is negative on the chemical examination. Two distinct forms of crystals are observed in the microscopic examination.
 - a. What type of crystals would you expect to be present?
 - b. What are the two crystal forms present?
 - c. Describe the two forms.
 - d. Which form would you expect to be predominant?

126 CHAPTER 6 • Microscopic Examination of Urine

Continued

6. A female patient comes to the outpatient clinic with symptoms of UTI. She brings a urine specimen with her. Results of the routine analysis performed on this specimen are as follows:

COLOR: Yellow
CLARITY: Hazy
SP. GRAVITY: 1.015
BILIRUBIN: Negative
UROBILINOGEN: Normal
PROTEIN: Negative
GLUCOSE: Negative

KETONES: Negative
BLOOD: Small
BILIRUBIN: Negative
UROBILINOGEN: Normal
NITRITE: Negative
LEUKOCYTE: 2+

Microscopic:

1 to 3 RBCs/hpf Heavy bacteria

8 to 10 WBCs/hpf Moderate squamous epithelial

cells

a. What discrepancies are present between the chemical and microscopic test results?

b. State a reason for the discrepancies.

c. Identify a chemical result in the urinalysis that confirms your reason for the discrepancies.

d. What course of action should the laboratory take to obtain accurate results for this patient?

7. A high-school student is taken to the emergency room with a broken leg that occurred during a football game. The urinalysis results are as follows:

COLOR: Dark yellow KETONES: Negative BLOOD: Small SP. GRAVITY: 1.030 BILIRUBIN: Negative

PROTEIN: 2+ UROBILINOGEN: Normal NITRITE: Negative LEUKOCYTE: Negative

Microscopic:

0 to 2 RBCs/hpf 0 to 4 hyaline casts/lpf 0 to 3 WBCs/hpf 0 to 3 granular casts/lpf

Few squamous epithelial cells

a. Are these results of clinical significance?

b. Explain the discrepancy between the chemical and microscopic blood results.

c. What is the probable cause of the granular casts?

8. As supervisor of the urinalysis section, you are reviewing results. State why or why not each of the following results would concern you.

a. The presence of waxy casts and a negative protein in urine from a 6-month-old girl

b. Increased transitional epithelial cells in a specimen obtained following cystoscopy

c. Tyrosine crystals in a specimen with a negative bilirubin test result

d. Cystine crystals in a specimen from a patient diagnosed with gout

e. Cholesterol crystals in urine with a specific gravity greater than 1.040

f. Trichomonas vaginalis in a male urine specimen

g. Amorphous urates and calcium carbonate crystals in a specimen with a pH of 6.0











Quality Assessment and Management in the Urinalysis Laboratory

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

- Discuss the quality assessment procedures and documentation for quality control of specimens, methodology, reagents, control materials, instrumentation, equipment, and reporting of results in the urinalysis laboratory.
- 2 Define the preanalytical, analytical, and postanalytical components of quality assessment.
- **3** Distinguish between the components of internal quality control, external quality control, and proficiency testing.
- 4 List the elements required for quality assurance as

- regulated by the Clinical Laboratory Improvement Amendments (CLIA).
- 5 Describe the four levels of the CLIA complexity model and how they relate to urinalysis testing.
- 6 Discuss the importance of continuous quality improvement and total quality management, including the recommendations of the Joint Commission on Accreditation of Healthcare Organizations.
- 7 Discuss the prevention of medical errors and the definition of a sentinel event.

KEY TERMS

accreditation
continuous quality
improvement
external quality control
internal quality control

process
proficiency testing
quality assessment
quality control

quality system
sentinel event
total quality management
turnaround time

The term *quality assessment* (QA) refers to the overall process of guaranteeing quality patient care and is regulated throughout the total testing system. *Quality system* refers to all of the laboratory's policies, processes, procedures, and resources needed to achieve quality testing.¹ In a clinical laboratory, a quality assessment program includes not only testing controls, referred to as *quality control* (QC), but also encompasses preanalytical factors (e.g., specimen collection,

handling, and storage), analytical factors (e.g., reagent and test performance, instrument calibration and maintenance, personnel requirements, and technical competence), postanalytical factors (e.g., reporting of results and interpretation), and documentation that the program is being meticulously followed. Included in a QA program are procedure manuals, *internal quality control* and *external quality control*, standardization, *proficiency testing* (PT), record keeping, equip-

128

ment maintenance, safety programs, training, education and competency assessment of personnel, and a scheduled and documented review process. Essentially, QA is the continual monitoring of the entire test process from test ordering and specimen collection through reporting and interpreting results. Written policies and documented actions as they relate to the patient, the laboratory, ancillary personnel, and the health-care provider are required. Having written remedial actions mandating the steps to take when any part of the system fails is essential to a QA program.

During the discussion of the routine urinalysis in the preceding chapters, the methods of ensuring accurate results were covered on an individual basis for each of the tests. Because QA in the urinalysis laboratory—or any other laboratory department—is an integration of many factors, this section will provide a collection of the procedures essential for providing quality urinalysis.

Documentation of QA procedures is required by all laboratory accreditation agencies, including the Joint Commission on the Accreditation of Healthcare Organizations (JCAHO), College of American Pathologists (CAP), American Association of Blood Banks (AABB), American Osteopathic Association (AOA), American Society of Histocompatibility and Immunogenetics (ASHI), and the Commission on Laboratory Assessment (COLA); it is also required for Medicare reimbursement. Guidelines published by CAP and the Clinical and Laboratory Standards Institute (CLSI)—previously called the National Committee for Clinical Laboratory Standards-provide very complete instructions for documentation and are used as a reference for the ensuing discussion of the specific areas of urinalysis OC and OA.2,3

Documentation in the form of a procedure manual is required in all laboratories, and this format is used as the basis for the following discussion.

Urinalysis Procedure Manual

A procedure manual containing all the procedures performed in the urinalysis section must be available for reference in the working area and must comply with the CLSI guidelines. The following information is included for each procedure: principle or purpose of the test, clinical significance, patient preparation, specimen type and method of collection, specimen acceptability and criteria for rejection, reagents, standards and controls, instrument calibration and maintenance protocols and schedules, step-by-step procedure, calculations, frequency and tolerance limits for controls and corrective actions, normal values and critical values, interpretation of results, specific procedure notes, limitations of the method, method validation, confirmatory testing, recording of results, references, effective date, author, and review schedule. Current package inserts should be reviewed and available at the workplace. Electronic manuals are acceptable and must be readily available to all personnel. As with written procedural manuals, electronic versions must be subjected to proper document control (i.e., only authorized persons may make changes, changes are dated/signed [manual or electronic], and there is documentation of periodic review).²

The evaluation of procedures and adoption of new methodologies is an ongoing process in the clinical laboratory. Whenever changes are made, the procedure should be reviewed and signed by a person with designated authority, such as the laboratory director or section supervisor (Fig. 7-1),

URINALYSIS SECTION SPECIMEN ACCEPTABILITY/LABELING Prepared by: Initial approval: Procedure placed in use: Revised: Reason for Revision: Effective Date Technical Approval Medical Director Approval Reviewed Reviewed Reviewed Reviewed Reviewed

Figure 7–I Example of procedure review documentation. (Adapted from the Department of Pathology, St. Joseph Hospital, Omaha, Nebr.)

and personnel should be notified of the changes. Documentation of an annual review of all procedures by the designated authority must also be substantiated.

Preanalytical Factors

Preanalytical factors are the variables that occur before the actual testing of the specimen and include test requests, patient preparation, specimen collection, handling, and storage. Health-care personnel outside the clinical laboratory control many of these factors, such as ordering tests and specimen collection. Communication between departments and adequate training on the correct procedures for ordering a test, collecting, and transporting the specimen improves the *turnaround time* (TAT) of results, avoids duplication of test orders, and ensures a high-quality specimen.

Specimen Collection and Handling

Specific information on specimen collection and handling should be stated at the beginning of each procedure listed in the manual. Requisition forms and computerized entry forms should designate the type of urine specimen to be collected and the date and time of collection. The form should include space for recording (1) the actual date and time of specimen collection, (2) whether the specimen was refrigerated before transporting, (3) the time the specimen was received in the laboratory and the time the test was performed, (4) tests requested, (5) an area for specific instructions that might affect the results of the analysis, and (6) patient identification information.³ The patient's sex, age or date of birth, and, when appropriate, the source of the specimen and the time it was collected, must be documented.¹

Patient preparation (e.g., fasting or elimination of interfering medications), type and volume of specimen required, and the need for sterile or opaque containers must be included with the specific procedure. All urine specimens should be examined within 2 hours. If this is not possible, written instructions for the preservation of the specimen must be available.

Instructions of a general nature, such as procedures for the collection of clean-catch and timed specimens, processing of specimens, and any printed materials given to patients, are also included in the manual.

Criteria for specimen rejection for both physical characteristics and labeling errors must be present. In Table 7–1, an example of a policy for handling mislabeled specimens is provided. Written criteria for rejection of specimens must be documented and available to the health-care provider and nursing staff.

Laboratory personnel must determine the suitability of a specimen and document any problems and corrective actions taken. An example of an internal laboratory quality improvement form is shown in Figure 7-2. It is used as a tool to document a problem at the point of discovery, describing what happened and the immediate corrective action taken. This

Table 7–1

Policy for Handling Mislabeled Specimens

Do NOT assume any information about the specimen or patient.

Do NOT relabel an incorrectly labeled specimen. Do NOT discard the specimen until investigation is complete.

Leave specimen EXACTLY as you receive it; put in the refrigerator for preservation until errors can be resolved. Notify floor, nursing station, doctor's office, etc. of problem and why it must be corrected for analysis to continue.

Identify problem on specimen requisition with date, time, and your initials.

Make person responsible for specimen collection participate in solution of problem(s). Any action taken should be documented on the requisition slip.

Report all mislabeled specimens to the quality assurance board.

From Schweitzer, SC, Schumann, JL, and Schumann, GB: Quality assurance guidelines for the urinalysis laboratory. Journal of Medical Technology 3(11): 568, 1986, with permission.

enables the laboratory director to capture the information to determine the root cause analysis and develop a preventive or corrective action plan. This documentation is required for reporting a sentinel event (described later in the chapter). Laboratory information systems have the capability to electronically generate these forms for review. An acceptable specimen requires verification of the patient's identification information on the requisition form and the container label, timely transport to the laboratory, the presence of refrigeration or recommended preservative if transport was delayed, and collection of an adequate amount of the correct urine specimen type in a noncontaminated, tightly closed container. After receipt in the laboratory, the specimen must be processed immediately or, if necessary, stored in a refrigerator and protected from light.³

Analytical Factors

The analytical factors are the processes that directly affect the testing of specimens. They include reagents, instrumentation and equipment, testing procedure, QC, *preventive maintenance* (PM), access to procedure manuals, and competency of personnel performing the tests.

Reagents

The manual should state the name and chemical formula of each reagent used, instructions for preparation, when necessary, or company source of prepared materials, storage requirements, and procedures for reagent QC. The type of water used for preparing reagents and controls must be spec-

	CONFIDENTIAL		
Instructions: Section I should be	e completed by the in	dividual identifying ti	he event.
ate of report:	Reported by:		
ate of incident:			
tient MR#	Patient accession	on#	
Section I Summary of incident — <i>describe what h</i>	appened		
What immediate corrective action was ta	iken?		
Provide the ORIGINAL to team leader/te Date: To: Forwarded for follow-up:	_ _	hin 24 hours of incide	ent discovery
To:	_		
To:	ecking #ed by laboratory man		ours
ction II. Management investigation: Transtructions: Section II should be complete. Check appropriate problem category Unacceptable patient sam (Due to hemolysis, QNS)	ecking #ed by laboratory man	agement within 72 h	ours
To: action II. Management investigation: Transtructions: Section II should be complete. Check appropriate problem category Unacceptable patient sam (Due to hemolysis, QNS) Equipment related event	ecking # ed by laboratory man ples , or contaminated)	Wrong tube t	ours ype sample
To:	ecking # ed by laboratory man ples , or contaminated) dure deviation	Wrong tube t Misidentified Wrong location	ours ype sample on
cction II. Management investigation: Transtructions: Section II should be complete. Check appropriate problem category Unacceptable patient same (Due to hemolysis, QNS) Equipment related event Standard operating proceed	ecking # ed by laboratory man ples , or contaminated) dure deviation	Wrong tube t	ours ype sample on
ction II. Management investigation: Transtructions: Section II should be complete. Check appropriate problem category Unacceptable patient same (Due to hemolysis, QNS) Equipment related event Standard operating proces	ecking # ed by laboratory man ples , or contaminated) dure deviation	Wrong tube t Misidentified Wrong location	ours ype sample on
ction II. Management investigation: Transtructions: Section II should be complete. Check appropriate problem category Unacceptable patient same (Due to hemolysis, QNS) Equipment related event Standard operating proceed Communication problem/c	ecking # ed by laboratory man ples , or contaminated) dure deviation	Wrong tube t Misidentified Wrong location	ours ype sample on
ction II. Management investigation: Transtructions: Section II should be complete. Check appropriate problem category Unacceptable patient same (Due to hemolysis, QNS) Equipment related event Standard operating process Communication problem/c	ecking # ed by laboratory man ples , or contaminated) dure deviation	Wrong tube t Misidentified Wrong location	ours ype sample on
ction II. Management investigation: Transtructions: Section II should be complete. Check appropriate problem category Unacceptable patient sam (Due to hemolysis, QNS) Equipment related event Standard operating proced Communication problem/c Accident Explain answers:	ed by laboratory man ples , or contaminated) dure deviation omplaint	Wrong tube t Misidentified Wrong location	ours ype sample on
ction II. Management investigation: Transtructions: Section II should be complete. Check appropriate problem category Unacceptable patient sam (Due to hemolysis, QNS) Equipment related event Standard operating proced Communication problem/c Accident Explain answers:	ed by laboratory man ples , or contaminated) dure deviation omplaint	Wrong tube t Misidentified Wrong location	ours ype sample on
ction II. Management investigation: Transtructions: Section II should be complete. Check appropriate problem category Unacceptable patient sam (Due to hemolysis, QNS) Equipment related event Standard operating proced Communication problem/c Accident Explain answers:	ed by laboratory man ples , or contaminated) dure deviation omplaint	Wrong tube t Misidentified Wrong location	ours ype sample on
ction II. Management investigation: Transtructions: Section II should be complete. Check appropriate problem category Unacceptable patient sam (Due to hemolysis, QNS) Equipment related event Standard operating proced Communication problem/c Accident Explain answers:	ed by laboratory man ples , or contaminated) dure deviation omplaint	Wrong tube t Misidentified Wrong location	ours ype sample on
To:	ed by laboratory man ples , or contaminated) dure deviation omplaint ations:	Wrong tube t Misidentified Wrong locati Other (explain	ours ype sample on n)
To:	ecking # ples , or contaminated) dure deviation omplaint	Wrong tube t Misidentified Wrong locati Other (explain	ours ype sample on n)
(Due to hemolysis, QNS) Equipment related event Standard operating proced Communication problem/c	ed by laboratory man ples , or contaminated) dure deviation omplaint ations:	Misidentified Wrong locati Other (explain	ours ype sample on n)

Figure 7–2 Sample of Quality Improvement Follow-up Report form. (From Danville Regional Medical Center Laboratory, Danville, Va., with permission.)

ified. Distilled or deionized water must be available. A boldtype statement of any safety or health precautions associated with reagents should be present. An example of this would be the heat produced in the Clinitest reaction.

All reagents and reagent strips must be properly labeled with the date of preparation or opening, purchase and received date, expiration date, and appropriate safety information. Reagent strips should be checked against known negative and positive control solutions on each shift or at a minimum once a day, and whenever a new bottle is opened. Reagents are checked daily or when tests requiring their use are requested. Results of all reagent checks are properly recorded.

Instrumentation and Equipment

Instructions regarding the operation, performance and frequency of calibration, limitations, and procedures to follow when limitations or linearity are exceeded, such as dilution procedures, must be clearly stated in the procedure manual. Instructions detailing the appropriate recording procedures must be included.

The most frequently encountered instruments in the urinalysis laboratory are refractometers, osmometers, automated reagent strip readers, and automated microscopy instruments. Refractometers are calibrated on each shift against distilled water (1.000) and a known control, such as 5% saline (1.022 \pm 0.001) or 9% sucrose (1.034 \pm 0.001). Two levels of commercial controls are available for the osmometer, urine reagent strip tests, and hCG kit tests. All control values must be recorded. Automated urinalysis systems and reagent strip readers are calibrated using manufacturer-supplied calibration materials following the protocol specified by the manufacturer. Both positive and negative control values must be run and recorded (Fig. 7-3). Evidence of corrective action for any failed QC tests must be documented. No patient's testing may be performed until QC is acceptable.

Equipment found in the urinalysis laboratory commonly includes refrigerators, centrifuges, microscopes, and water baths. Temperatures of refrigerators and water baths should be taken daily and recorded. Calibration of centrifuges is customarily performed every 3 months, and the appropriate relative centrifugal force for each setting is recorded. Centrifuges are routinely disinfected on a weekly basis. Microscopes should be kept clean at all times and have an annual professional cleaning. A routine PM schedule for instruments and equipment should be prepared as mandated by JCAHO or CAP guidelines, and records are kept of all routine and nonroutine maintenance performed.

Deionized water used for reagent preparation is quality controlled by checking pH and purity meter resistance on a weekly basis and the bacterial count on a monthly schedule. All results must be recorded on the appropriate forms.

Testing Procedure

Detailed, concise testing instructions are written in a step-bystep manner. Instructions should begin with specimen preparation, such as time and speed of centrifugation, and include types of glassware needed, time limitations and stability of specimens and reagents, calculation formulas and a sample calculation, health and safety precautions, and procedures. Additional procedure information including reasons for special precautions, sources of error and interfering substances, helpful hints, clinical situations that influence the test, alternative procedures, and acceptable TATs for STAT tests are listed under the title of Procedure Notes following the step-by-step procedure.⁴

Reference sources should be listed. Manufacturer's package inserts may be included but cannot replace the written procedure. The laboratory director must sign and date new procedures and all modifications of procedures before they are used.¹

Quality Control

Quality control refers to the materials, procedures, and techniques that monitor the accuracy, precision, and reliability of a laboratory test.⁵ QC procedures are performed to ensure that acceptable standards are met during the process of patient testing. Specific QC information regarding the type of control specimen preparation and handling, frequency of use, tolerance levels, and methods of recording should be included in the step-by-step instructions for each test. QC is performed at scheduled times, such as at the beginning of each shift or prior to testing patient samples, and it must always be performed if reagents are changed, an instrument malfunction has occurred, or if test results are questioned by the physician. Control results must be recorded in a log, either paper or electronic. Patient test results may not be reported until the QC is verified. Both external quality control monitoring and internal quality control processes are practiced in the urinalysis laboratory.

External Quality Control Monitoring

External quality controls are used to verify the accuracy (ability to obtain the expected result) and precision (ability to obtain the same result on the same specimen) of a test and are exposed to the same conditions as the patient samples. Reliability is the ability to maintain both precision and accuracy. Commercial controls are available for the urine chemistry tests, specific gravity, and for certain microscopic constituents. Analysis of two levels of control material is required. Documentation of QC includes dating and initialing the material when it is first opened and recording the manufacturer's lot number and the expiration date each time a control is run and the test result is obtained. Food and Drug Administration (FDA) standards require that control material test negative for HIV and hepatitis B virus. External controls are tested and interpreted in the laboratory by the same person performing the patient testing.

Control data are evaluated prior to release of patient results. Data obtained from repeated measurements have a gaussian distribution or spread in the values that indicate the ability to repeat the analysis and obtain the same value. The 132

QUALITY CONTROL

			Positive control				Negative control													
			GLU	BIL	KET	SP	BLD	PH	PROT	NIT	LEU	GLU	BIL	KET	SP	BLD	PH	PROT	NIT	LEU EST
			mg/dl		mg/dl	GR 1 0			mg/dl		EST	mg/dl		ma/dl	GR 1 0			ma/dl		EST
Control value	25		mg/ui		mg/ui	1.0			mg/ui			mg/ui		mg/ui	1.0			mg/ui		
Reagent Lot # EXP	TECH	DATE																		
Lot #																				
EXP																				

Figure 7–3 Sample instrument QC recording sheet. (Adapted from the Department of Pathology, Methodist Hospital, Omaha, Nebr., with permission.)

laboratory, after repeated testing, establishes the value for each analyte, and the mean and standard deviation is calculated. The *control mean* is the average of all data points and the *standard deviation* (SD) is a measurement statistic that describes the average distance each data point in a normal distribution is from the mean. The *coefficient of variation* (CV) is the SD expressed as a percentage of the mean. The CV indicates whether the distribution of values about the mean is in a narrow versus broad range and should be less than 5%. Confidence intervals are the limits between which the specified proportion or percentage of results will lie. *Control ranges* are determined by setting confidence limits that are within ± 2 SD or ± 3 SD of the mean, which indicates that 95.5% to 99.7% of the values are expected to be within that range.

Values are plotted on Levy-Jennings control charts to visually monitor control values. Immediate decisions about patient results are based on the ability of control values to remain within a preestablished limit. Changes in accuracy of results are indicated by either a *trend* that is a gradual changing in the mean in one direction or a *shift* that is an abrupt change in the mean (Fig. 7-4). Changes in precision are shown by a large amount of scatter about the mean and an uneven distribution above and below the mean that are most often caused by errors in technique.

Month:

20

Corrective action, including the use of new reagents, reagent strips, or controls, and the verification of lot numbers and expiration dates, must be taken when control values are outside the tolerance limits. All corrective actions taken are

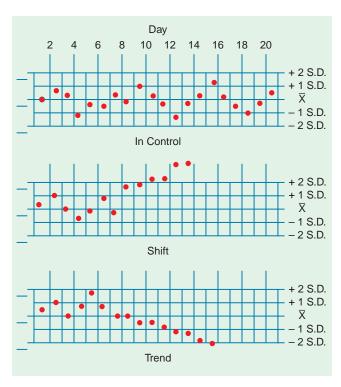


Figure 7–4 Levy-Jennings charts showing in-control, shift, and trend results.

documented. A protocol for corrective action is shown in Figure 7-5. A designated supervisor reviews all QC results.

Laboratories may participate in a commercial QC program. Results from the same lot of QC material sent by the manufacturer to participating laboratories are returned to the manufacturer for statistical analysis and comparison with other laboratories using the same methodology.

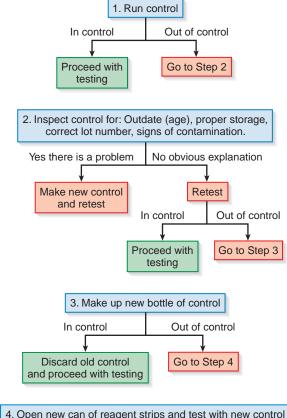
Internal Quality Control Monitoring

Internal quality control consists of internal monitoring systems built in to the test system and may be called electronic, internal, or procedural controls. Internal or procedural controls monitor the correct addition of a patient specimen or reagent, the instruments/reagents interaction, and test completion. Electronic controls monitor a test system's electronic or electric components.

Proficiency Testing

PT is the testing of unknown samples received from an outside agency. It provides unbiased validation of the quality of patient test results. Several commercial vendors provide proficiency testing. CAP, the American Association of Bioanalysts (AAB), American Proficiency Institute (API), Accutest, American Academy of Family Physicians (AAFP), and the Wisconsin State Laboratory of Hygiene (WSLH) are a few of the most common proficiency testing vendors. Laboratories subscribing to these programs receive lyophilized or ready-to-use specimens for routine urinalysis and Kodachromes or color plates for sediment constituent identification. The results are returned to the proficiency testing vendors, where they are statistically analyzed with those from

- A. Record all actions taken and the resolution of any problems
- B. Use the flow diagram below:



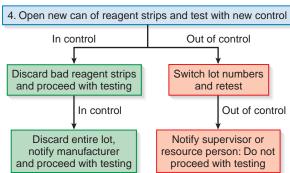


Figure 7–5 "Out-of-control" procedures. (From Schweitzer, SC, Schumann, JL, and Schumann, GB: Quality assurance guidelines for the urinalysis laboratory. Journal of Medical Technology 3(11): 567-572, 1986, with permission.)

all participating laboratories, and a report is returned to the laboratory director. The laboratory accuracy is evaluated and compared with other laboratories using the same method of analysis. Corrective action must be taken for unacceptable results.

Personnel and Facilities

Quality control is only as good as the personnel performing and monitoring it. Personnel must understand the importance of QA, and the program should be administered in a manner such that personnel view it as a learning experience rather than as a threat. Up-to-date reference materials and atlases should be readily available, and documentation of continuing education must be maintained.

An adequate, uncluttered, safe working area is also essential for both quality work and personnel morale. Standard precautions for handling body fluids must be followed at all times.

Postanalytical Factors

Postanalytical factors are processes that affect the reporting of results and correct interpretation of data.

Reporting of Results

Standardized reporting formats and, when applicable, reference ranges should be included with each procedure covered in the procedure manual. A written procedure for reporting, reviewing, and correcting errors must be present.

Forms for reporting results should provide adequate space for writing and should present the information in a logical sequence. Standardized reporting methods minimize health-care provider confusion when interpreting results (Fig. 7-6).

Electronic transmission is now the most common method for reporting results. Many urinalysis instruments have the capability for the operator to transmit results directly

from the instrument to the designated health-care provider. It is essential that the operator carefully review results before transmittal. Results may also be manually entered into the laboratory computer system and then transmitted to the health-care providers.

Erroneous results must be corrected in a timely manner to assure that the patient does not receive treatment based on incorrect results. Errors can occur in patient identification, specimen labeling, or result transcription. The patient's record should be corrected as soon as the error is detected; however, the original result must not be erased in the event that the health-care provider treated the patient based on the erroneous results. Appropriate documentation of erroneous results should follow institutional protocol.

Written procedures should be available for the reporting of critical values (Fig. 7-7). In laboratories analyzing pediatric specimens, this should include the presence of ketones or reducing substances in newborns.

Interpretation of Results

The specificity and the sensitivity for each test should be included in the procedure manual for correct interpretation of results. All known interfering substances should be listed for evaluation of patient test data. A well-documented QA program ensures quality test results and patient care.

```
MICROSCOPIC QUANTITATIONS
Quantitate an average of 10 representative fields. Do not quantitate budding yeast,
mycelial elements, trichomonas, or sperm, but do note their presence with the
appropriate LIS code.
 Epithelial cells/LPF
         None:
                            0
                            0-5
         Rare:
         Few:
                            5-20
                            20-100
         Moderate:
         Many:
                            >100
 Casts/LPF
         None:
         Numerical ranges: 0-2, 2-5, 5-10, >10
 RBCs/HPF
         Numerical ranges: 0-2, 2-5, 5-10, 10-25, 25-50, 50-100, >100
WBCs/HPF
         Numerical ranges: 0-2, 2-5, 5-10, 10-25, 25-50, 50-100, >100
 Crystals/HPF
                            0
         None:
                            0-2
         Rare:
         Few:
                            2-5
         Moderate:
                            5-20
         Many:
                            >20
 Bacteria/HPF
         None:
                            0-10
         Rare:
         Few:
                            10-50
                            50-200
         Moderate:
                            >200
         Many:
 Mucous threads
                            0-1
         Rare:
         Few:
                            1-3
         Moderate:
                            3-10
         Many:
                            >10
```

Figure 7–6 Sample standardized urine microscopic reporting format. (From University of Nebraska Medical Center, Omaha, Nebr., with permission.)

Poor testing technique

POSITIVE KETONES:

All positive ketones on pediatrics less than or equal to two years old shall be called to the appropriate nursing unit. The time of the call, initials of the "tech", and the name of the person receiving the call are to be documented in the computer as a chartable footnote appended to the result.

POSITIVE CLINITEST:

All positive Clinitest results on pediatrics less than or equal to two years old shall be called to the appropriate nursing unit. The time of the call, initials of the "tech", and the name of the person receiving the call are to be documented in the computer as a chartable footnote appended to the result

Figure 7–7 Sample critical results—reporting procedure. (Adapted from the Department of Pathology, St. Joseph Hospital, Omaha, Nebr., with permission.)

■ ■ ■ Regulatory Issues

Clinical Laboratory Improvement Amendments '88 (CLIA' 88) stipulate that all laboratories that perform testing on human specimens for the purposes of diagnosis, treatment, monitoring, or screening must be licensed and obtain a certificate from the CLIA program that corresponds to the complexity of tests performed. This includes all independent and hospital laboratories, physician-office laboratories, rural health clinics, mobile health screening entities such as health fairs, and public health clinics. CLIA defined categories of diagnostic laboratory tests based on the test complexity and risk factors related to erroneous test results and specified the training and educational levels required of personnel performing the tests. Tests are assigned to the following categories: waived, provider-performed microscopy, moderate complexity, and high complexity. Nonwaived testing replaces the terms "moderate" and "high complexity" testing when referring to requirements that pertain to both levels of testing.1

Waived tests are considered easy to perform and interpret, require no special training or educational background,

Summary of Quality Assurance Errors Preanalytical Instrument malfunction Patient misidentification Interfering substances present Wrong test ordered Misinterpretation of quality Incorrect urine specimen control data type collected Postanalytical Insufficient urine volume Patient misidentification Delayed transport of urine to the laboratory Poor handwriting Incorrect storage or Transcription error preservation of urine Poor quality of instrument printer Analytical Sample misidentification Failure to send report Failure to call critical values Erroneous instrument calibration Reagent deterioration Inability to identify interfering substances

require only a minimum of standardization and QC, and are not considered critical to immediate patient care. Urinalysis tests in this category are manual dipstick/chemical tablet testing, microalbumin, ovulation detection, urine toxicology, and urine pregnancy tests. The list of tests is rapidly growing, as many different manufacturers continue to modify tests, enabling them to be approved for waived testing. An updated list of tests is available at www.cms.hhs.gov/clia.

A modification of the CLIA categories created a new certificate category for provider-performed microscopy (PPM) procedures. This category includes certain microscopic procedures that can be performed in conjunction with any waived test to avoid disruption in the patient visit. Personnel standards authorize only physicians, physician's assistants, nurse practitioners, and dentists to perform the tests. Laboratories performing PPM must meet the moderate-complexity requirements for proficiency testing, patient test management, QC, and QA. Urine sediment examinations, wet mounts, and KOH preparations are examples of the tests in this category. A complete listing is provided in Table 7–2.

Moderate-complexity tests are more difficult to perform than are waived tests and require documentation of training in testing principles, instrument calibration, periodic profi136

Table 7–2

Provider-Performed Microscopy Category

Urine sediment examination

Wet mounts (vaginal, cervical, skin, or prostatic secretions)

KOH preparations

Pinworm examinations

Fern test

Postcoital direct, qualitative examinations of vaginal mucus

Fecal leukocyte examination

Qualitative semen analysis

Nasal smear for granulocytes

ciency testing and on-site inspections. In a hospital setting, even waived tests must adhere to the moderate-complexity test standards. Most chemistry and hematology tests are assigned to this category. Automated or semiautomated urinalysis tests and urine microscopic procedures are considered moderate-complexity tests.

High-complexity tests require sophisticated instrumentation and an increased level of interpretation by the testing personnel. Many tests performed in microbiology, immunology, immunohematology, and cytology are in this category.

CLIA regulations specify required components for QA that include patient test management assessment, QC assessment, proficiency testing assessment, comparison of test results, relationship of patient information to patient test results, patient confidentiality, specimen identification and integrity, personnel competency, personnel assessment, communications, complaint investigation, QA review with staff, and QA records.⁸

Patient test management includes systems for patient preparation, correct specimen collection, sample identification, sample preservation, sample transportation, sample processing, and accurate result reporting. There must be verification that the laboratory has a system in place for monitoring and evaluating confidentiality of patient information. The testing facility must have available written procedures for each system to ensure that specimen integrity and identification are maintained throughout the entire testing process.

Quality control assessment requires that quality control records include date, results, testing personnel, and lot numbers for reagents and controls. Records must be retained for 2 years. Records should be reviewed daily and monthly to detect trends, shifts, inconsistent test systems, or operator difficulties.

Proficiency testing is required for all laboratories performing moderate-complexity or high-complexity testing (nonwaived testing). An approved program for regulated tests involves three events per year with five challenges per analyte that is regulated.⁸ For nonregulated tests, accuracy must be verified two times per year. Samples must be tested in the

same manner as patient samples. Communication or consultation with other laboratories is not permitted.

Personnel assessment includes education and training, continuing education, competency assessment, and performance appraisals. Each new employee must have documentation of training during orientation to the laboratory. A checklist of procedures must be documented with the date and initials of the person doing the training and of the employee being trained.

The qualifications of the personnel performing patient care are also regulated to ensure that only persons with appropriate education and training perform procedures. Healthcare personnel become certified and/or licensed in their particular fields through the completion of specified educational requirements and/or satisfactory performance on standardized proficiency examinations. The level of education is documented in the employee personnel file. A record of all continuing education sessions should be kept in each personnel file. Currently no minimum hours of continuing education are mandated.

Technical competency assessment as mandated by CLIA must be done for each employee for each procedure twice during the first year of employment and then annually. Methods for assessing competency include direct observation, review of QC records, review of proficiency testing records, and written assessments.^{8,9}

Performance appraisals for each employee are done following the institution's protocol and evaluate the standards of performance as designated by the job description. The standards must be specific and measurable and may include evaluation of attitude as well as organizational and communication skills.

Clinical laboratory records must be maintained for 2 years. These records include patient test results, QC data, reagent logs, test method verification, proficiency test data, competency assessment, education and training, equipment maintenance, service calls, documentation of problems, complaints, communication, inspection files, and certification records.

CLIA are jointly administered by the Centers for Medicare and Medicaid Services (CMS), FDA, and CDC. Accrediting agencies that have been approved by the federal government after demonstrating equivalency with CLIA standards include COLA (which is popular with physician office laboratories), the JCAHO, CAP (which serves large laboratories), AOA, AABB, and ASHI. Compliance with accreditation regulations is ensured by periodic on-site visits to facilities by inspection teams and through performance on proficiency tests. If deficiencies are present, the facility must correct them within a specified time and be reinspected. Waived and PPM laboratories are not subject to routine inspection. Inspections must be scheduled and are done within the first 2 years of certification. The QA requirements are regulated to emphasize the importance of assessing quality throughout the total testing process. The ultimate goal of these agencies is to promote continuous quality improvement (CQI).9

Quality Management

Quality control and quality assessment are part of institutional quality management programs. CQI, Improving Organizational Performance (IOP), total quality management (TQM), and Six Sigma are all programs that evolved from the Deming quality management philosophy, each with a slightly different emphasis. Whereas QA is designed to maintain an established level of quality, TQM and CQI are designed to develop methods to continually improve the quality of health care. Standards from the JCAHO address this concept by requiring documentation showing that effective, appropriate patient care is being provided, as shown by positive patient outcomes. Areas addressed by the standards include availability of services, timeliness, continuity of care, effectiveness and efficiency of services, safety of service provided, and respect and care by the personnel providing services.

TQM is based on a team concept involving personnel at all levels working together to achieve a final outcome of customer satisfaction through implementation of policies and procedures identified by the CQI program. This concept applies scientific principles to management and uses graphical and statistical analysis of data as a basis for decision making. 10 TQM is a systematic problem-solving approach using visual tools to identify the steps in the process for meeting customer satisfaction of quality care in a timely manner at reduced costs. In the health-care setting, the patient is the ultimate customer; customers also include health-care providers, personnel in other departments, and the patient's family and friends. TQM is far-reaching and encompasses the quality and performance assessment of the infrastructure (physical, personnel, and management), processes, outcomes, and customer satisfaction.

The focus of CQI is to improve patient outcomes by providing continual quality care in a constantly changing health-care environment. Group problem solving and teamwork are elements to support the identification and resolution of problems across different departments. Helpful tools to assess CQI are flowcharts, cause-and-effect diagrams (fishbone diagrams), pareto charts, histograms, run charts, control charts, and scatter diagrams. A flowchart is a picture of the process mapping out each individual step so that each group

member can understand how it works. Cause-and-effect diagrams determine the cause of a problem and identify the different elements that contribute to the problem. They relate the interaction between equipment, methods, and customers. Pareto charts are based on the Pareto principle, which states that 80% of the trouble comes from 20% of the problems. Pareto charts are used to mainly identify the problems. The information in this type of graph displays the major contributors to a problem in descending order of importance. A run chart tracks individual data points recorded in a time sequence and compares the points to the average. It is useful to determine cyclic or seasonal differences. Control charts provide statistically determined limits drawn on both sides of the line indicating deviations from the average. Scatter diagrams are a visual plotting technique used to evaluate causeand-effect correlations between two variables. Histograms display the shape of distribution of a variable indicating the amount of variation and are often used to summarize and communicate data.

Many models based on W. Edward Deming's 14 principles of CQI are available for implementing CQI. The most widely used plan for quality improvement in health care is the Plan-Do-Check-Act (PDCA) strategy also known as the Plan-Do-Study-Act (PDSA) cycle. ¹¹

The "Plan" step is the process of making a change by identifying the customers and customer expectations, describing the current process, measuring and analyzing, focusing on improvement opportunities, identifying the root cause, and generating a solution. External customers are people such as the vendors or health-care providers who are not employed by one's organization. Internal customers are employees within the organization who are dependent on one's service. A nurse requesting a urinalysis result on a patient would be a customer of the laboratory. Customer needs and expectations are identified in the health-care field most often through complaint analysis, focus groups and interviews, satisfaction surveys, and JCAHO professional standards. An example would be "How to reduce the TAT for a patient's urinalysis test result?" Through the use of flowcharts, cause-and-effect diagrams, and pareto charts (Figs. 7-8 to 7-10), the data can visually be measured and analyzed, and the committee can arrive at a problem statement and focus on improvement

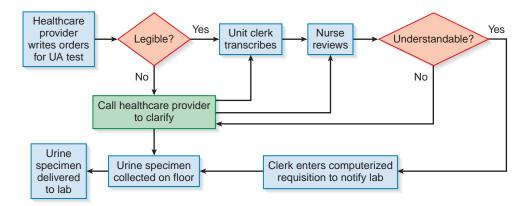


Figure 7–8 Flowchart demonstrating steps in the urinalysis collection procedure.

138 CHAPTER 7 • Quality Assessment and Management in the Urinalysis Laboratory

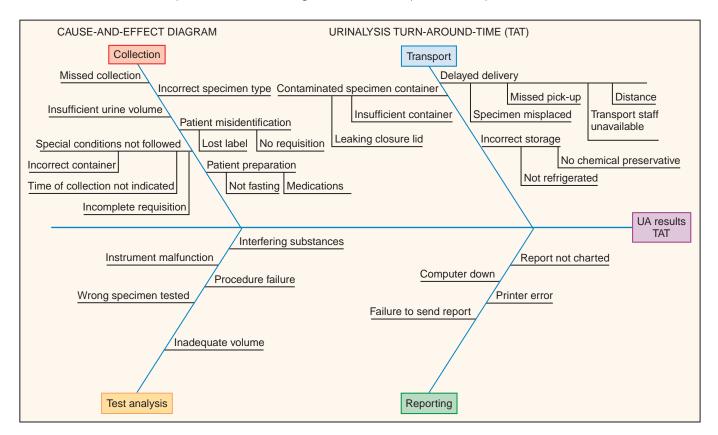


Figure 7–9 Cause-and-effect diagram for analyzing urinalysis TAT.

opportunities. After generating theories of causes and collecting data, the root cause can be pinpointed, and, through focus groups with customers, discussions with staff, and brainstorming, a solution can be produced.

The "Do" step is the process of testing the improvement by mapping out a trial run, implementing that run, collecting data, and analyzing the data. The person responsible for each step and the dates and time frames for the trial should be specified. Steps used to monitor implementation of the trial run and verification of results must be documented. As an example for the above urinalysis TAT, a trial run to shorten urinalysis testing TAT could be to transport the specimen

from the patient location to the laboratory via a pneumatic tube system immediately upon collection.

The "Check" or "Study" step involves evaluating the results and drawing conclusions as to the effect of the change. Tools to evaluate the solution are control charts, run or trend charts, simple observations, and surveys. From these results, it can be determined whether the process was a success, failure, or in need of minor modifications. An example would be to use a run chart to plot TATs from the time of collection of urine through the testing procedure to the time the report appeared in the patient chart for specimens being transported by the pneumatic tube system.

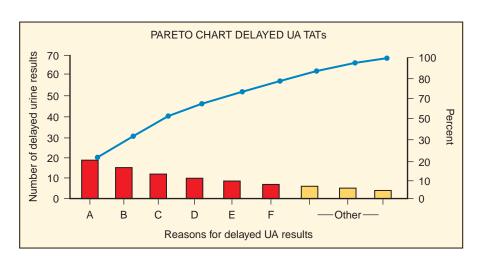


Figure 7–10 Pareto chart demonstrating causes of delayed urinalysis reporting.

The "Act" step is standardizing the change by modifying the standard procedure, policies, and performance expectations to reflect the changed process. These changes must be communicated effectively to the customers to ensure implementation and to avoid resistance to change. A plan must indicate ways the new procedure will be incorporated and how the customers will be supported throughout the change process, and it must provide training to the people involved. In the previous urinalysis example, proper training on bagging the specimen to avoid leakage and on operating the pneumatic tube system would be necessary. Implementation of a regular schedule of measurement to monitor the change over an extended period confirms the success of the change or the gain.

The JCAHO 1996 Comprehensive Accreditation Manual for Hospitals recommends a method for improving organizational performance (IOP) through work and management processes for the various departments of an organization to work together. Known as **PDMAI**, the plan provides standards PI.1 through PI.5 (plan, design, measure, assess, and improve) to outline a specific cycle for improving performance. 12–14 The five essential elements for performance improvement are as follows:

- **Plan (PI.1):** The hospital has a planned, systematic, hospital-wide approach to process design and performance measurement, assessment, and improvement
- Design (PI.2): New processes are designed well.
- **Measure (PI.3):** The organization has a systematic process in place to collect data.
- **Assess (PI.4):** The hospital uses a systematic process to assess collected data.
- **Improve (PI.5):** The hospital systematically improves its performance.

Other models such as the Six Sigma Quality Management emphasize a more quantitative methodology utilizing power function graphs, critical-error graphs, and OPSpecs charts. By instituting quality improvement methodologies, a health-care institution can develop a structured standardized format to systematically assess and document the quality of services to the customer.

Medical Errors

In November 1999, the National Academy of Sciences' Institute of Medicine (IOM) issued a report entitled "To Err is Human: Building a Safer Health System." The report stimulated considerable public and governmental concern by stating that the majority of adverse medical events were caused by preventable medical errors. Health-care institutions, accrediting agencies, and government agencies are placing increased emphasis on the designing of safe medical practices. The IOM report stresses that most medical errors are system related and are not caused by individual negligence or misconduct. Therefore, systems should be

designed to make it easy to do the right thing and hard to do the wrong thing.

The JCAHO has issued a new standard referred to as "Sentinel Event Policies and Procedures" requiring reporting of sentinel events. A sentinel event is defined as any unanticipated death or major permanent loss of function not related to the natural course of the patient's illness or underlying condition. Reportable events are suicide during institutional care, infant abduction or discharge to the wrong family, rape during institutional care, hemolytic transfusion reactions from major incompatibilities, and surgery on the wrong patient or body part. Sentinel events must be reported to the JCAHO within 45 days of the event. The report must include a root cause analysis and an action plan. Acceptable root cause analyses identify basic or causal factors that underlie variation in performance and focus primarily on systems and processes rather than individual performance. As the JCAHO analyzes sentinel event reports, it periodically publishes lists of specific sentinel event causes to alert the health-care community of areas to evaluate in their institution. 15

References

- 1. Centers for Medicare & Medicaid Services, Department of Health and Human Services: Clinical Laboratory Improvement Amendments, Updated Regulations, Brochure #1, How do they affect my laboratory? http://www.cms.hhs.gov/CLIA/05_CLIA_Brochures.asp Accessed November 2006.
- College of American Pathologists: Commission on Laboratory Accreditation, Urinalysis Checklist. College of American Pathologists, Skokie, Ill., 2005.
- 3. Clinical and Laboratory Standards Institute, NCCLS-Approved Guideline: GP16-A2, Vol 21 No. 19, Urinalysis and Collection, Transportation, and Preservation of Urine Specimens: Approved Guideline, 2001.
- Strasinger, SK: Urinalysis and Body Fluids, 3rd ed. FA Davis, Philadelphia, 1994.
- 5. Hodnett, J. Proficiency testing, we all do it—but what do the results mean? Lab Med 30(5):316-323, 1999.
- Centers for Medicare & Medicaid Services, Department of Health and Human Services: Clinical Laboratory Improvement Amendments, Brochure #4: Equivalent Quality Control Procedures. http://www.cms.hhs.gov/CLIA/05_CLIA_Brochures.asp Accessed November 2006.
- Schweitzer, SC, Schumann, JL, and Schumann, GB: Quality assurance guidelines for the urinalysis laboratory. J Med Technol 3(11):567-572, 1986.
- Centers for Medicare and Medicaid Services, Department of Health and Human Services: Current Clinical Laboratory Improvement Amendments Regulations and Guidelines. http://www.cms.hhs.gov/CLIA/Accessed December 2006.
- Costaras, J: Urinalysis: It gets no respect. ADVANCE for Medical Laboratory Professionals, pp. 10, 11, August 11, 1997.
- Yablonsky, MA: Total quality management in the laboratory from under the microscope into practice. Lab Med 26(4): 253-260, 1995.
- Hrdlicka, D: Quality Improvement Made Simple. Advanced Management Information Technology—3, Add-A Competency, University of Nebraska Medical Center, Division of Medical Technology, 1998.
- Comprehensive Accreditation Manual for Hospitals: The Official Handbook, Improving Organization Performance. CAMH Update 4, November 1997.

140 CHAPTER 7 • Quality Assessment and Management in the Urinalysis Laboratory

- 13. Holmes, R: Conquering performance improvement documentation for JCAHO. Medical Laboratory Observer 30(6):18, 19, 22, 24, 1998.
- Trant, C, Broda, K, and Edwards, G: Department of Pathology Duke University Medical Center, Durham, NC, JCAHO Inspection: Preparing the Laboratory, AACC 50th Anniversary Meeting Workshop 2407, Chicago, Ill., August 5, 1998.
- 15. Strasinger, SK, and Di Lorenzo, MS: The Phlebotomy Workbook, 2nd ed. FA Davis, Philadelphia, 2003.

QUESTIONS STUDY

- 1. Quality assessment refers to:
 - A. Analysis of testing controls
 - B. Increased productivity
 - C. Precise control results
 - D. Quality of specimens and patient care
- **2.** During laboratory accreditation inspections, procedure manuals are examined for the presence of:
 - A. Critical values
 - B. Procedure references
 - C. Procedures for specimen preservation
 - D. All of the above
- 3. Urinalysis procedure manuals are reviewed:
 - A. By the supervisor on each shift
 - B. Weekly by the pathologist
 - C. Only when a procedure is changed
 - D. Annually by a designated authority
- **4.** As supervisor of the urinalysis laboratory, you have just adopted a new procedure. You should:
 - A. Put the package insert in the procedure manual
 - B. Put a complete, referenced procedure in the manual
 - C. Notify the microbiology department
 - D. Put a cost analysis study in the procedure manual
- **5.** Indicate whether each of the following would be considered a 1) preanalytical, 2) analytical, or 3) postanalytical factor by placing the appropriate number in the space:

	Reagent expiration date
	Rejection of a contaminated specimen
	Construction of a Levy-Jennings chart
	Telephoning a positive Clinitest result on
	newborn
	Calibrating the centrifuge
	Collecting a timed urine specimen
Daiani	ized water used for the preparation of reason

- **6.** Deionized water used for the preparation of reagents should be checked for:
 - A. Calcium content
 - B. Bacterial content
 - C. Filter contamination
 - D. pH, purity, and bacteria

- 7. Would a control sample that has accidentally become diluted produce a trend or a shift in the Levy-Jennings plot?
 - A. Trend
 - B. Shift
- **8.** A color change that indicates when a patient's specimen or reagent is added correctly would be an example of:
 - A. External QC
 - B. Equivalent QC
 - C. Internal QC
 - D. Proficiency testing
- **9.** What steps are taken when the results of reagent strip QC are outside of the stated confidence limits?
 - A. Check the expiration date of the reagent strip
 - B. Run a new control
 - C. Open a new reagent strips container
 - D. All of the above
- **10.** When a new bottle of qc material is opened, what information is placed on the label?
 - A. The supervisor's initials
 - B. The lot number
 - C. The date and the laboratory worker's initials
 - D. The time the bottle was opened
- 11. When a control is run, what information is documented?
 - A. The lot number
 - B. Expiration date of the control
 - C. The test results
 - D. All of the above
- **12.** State which of the CLIA categories is assigned to each of the following laboratory tests by placing the appropriate number in front of the test.
 - 1. Waived
 - 2. PPM
 - 3. Moderate complexity
 - 4. High complexity
 - ____A. Reagent strip urinalysis
 - ____B. Urine culture
 - ____C. Complete urinalysis using the Clinitek 500
 - ____D. Urine microscopic
 - ___E. Urine pregnancy test
- **13**. How often does CLIA' 88 require documentation of technical competency?
 - A. Every 6 months
 - B. Once a year
 - C. Twice the first year and then annually
 - D. Twice the first year and then every 5 years
- 14. Who are the laboratory's "customers" in CQI?
 - A. Physicians
 - B. Patients' family members
 - C. Patients
 - D. All of the above

- 15. What is the primary goal of TQM?
 - A. Increased laboratory productivity
 - B. Improved patient outcomes
 - C. Reliability of test results
 - D. Precise test results
- **16.** Match the purpose for developing each of the following:
 - 1. Flowcharts
 - 2. Cause-and-effect diagrams
 - 3. Pareto charts
 - 4. Run charts
 - ___A. Determine cyclic and seasonal differences compared to an average
 - ____B. Break down a process into steps
 - ____C. Identify the major contributors to a problem
 - ____D. Determine the cause of a problem
- **17.** *True or False:* Most medical errors are the fault of individuals, not the system.

Case Studies and Clinical Situations

- **1.** State a possible reason for an accreditation team to report a deficiency in the following situations:
 - a. The urine microscopic reporting procedure has been recently revised.
 - b. An unusually high number of urine specimens are being rejected because of improper collection.
 - c. A key statement is missing from the clinitest procedure.
 - d. Open control bottles in the refrigerator are examined.

- **2.** A physician consults a medical technologist to answer the following questions regarding CLIA regulations:
 - a. Can I perform urine microscopics?
 - b. If I purchase an automated urinalysis strip reader and a chemistry analyzer:

Will my CLIA status be affected?

Will my office be required to perform proficiency testing?

Will my office be subject to COLA inspections?

- A hospital laboratory outreach coordinator is asked to develop a method to decrease the number of rejected specimens for urinalysis received from physicians' offices.
 - a. What accepted process could the coordinator follow?
 - b. Briefly outline the steps the coordinator should take to address this problem, including the use of visual documentation.
- **4.** As the new supervisor of the urinalysis section, you encounter the following situations. Explain whether you would accept them or take corrective action.
 - a. You are told that the supervisor always performs the cap proficiency survey.
 - b. QC is not performed daily on the clinitest tablets.
 - c. The urinalysis section is primarily staffed by personnel assigned to other departments for whom you have no personnel data.











8 CHAPTER

Renal Disease

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

- 1 Differentiate among renal diseases of glomerular, tubular, interstitial, and vascular origin.
- 2 Describe the processes by which immunologic damage is produced to the glomerular membrane.
- **3** Define glomerulonephritis.
- 4 Describe the characteristic clinical symptoms, etiology, and urinalysis findings in acute post-streptococcal and rapidly progressive glomerulonephritis, Goodpasture syndrome, Wegener's granulomatosis, and Henoch-Schönlein purpura.
- 5 Name a significant urinary sediment constituent associated with all of the aforementioned disorders
- 6 Name three renal disorders that also involve acute respiratory symptoms.
- 7 Differentiate between membranous and membranoproliferative glomerulonephritis.
- 8 Discuss the clinical course and significant laboratory results associated with immunoglobulin A nephropathy.

- **9** Relate laboratory results associated with the nephrotic syndrome to the disease process.
- 10 Compare and contrast the nephrotic syndrome and minimal change disease with regard to laboratory results and course of disease.
- 11 State two causes of acute tubular necrosis.
- 12 Name the urinary sediment constituent most diagnostic of renal tubular damage.
- 13 Describe Fanconi syndrome, Alport syndrome, and renal glucosuria.
- 14 Differentiate between diabetic nephropathy and nephrogenic diabetes insipidus.
- 15 Compare and contrast the urinalysis results in patients with cystitis, pyelonephritis, and acute interstitial nephritis.
- 16 Differentiate among causes of laboratory results associated with prerenal, renal, and postrenal acute renal failure.
- 17 Discuss the formation of renal calculi, composition of renal calculi, and patient management techniques.

KEY TERMS

antiglomerular basement membrane antibody cystitis

glomerulonephritis lithiasis nephrotic syndrome pyelonephritis tubulointerstitial disease

144 CHAPTER 8 • Renal Disease

Disorders throughout the body can affect renal function and produce abnormalities in the urinalysis. Considering that the major function of the kidneys is filtration of the blood to remove waste products, it becomes evident that the kidneys are consistently exposed to potentially damaging substances.

Renal disease is often classified as being glomerular, tubular, or *interstitial*, based on the area of the kidney primarily affected. In this chapter, the most commonly encountered disorders will be covered in relation to the affected areas of the kidney, keeping in mind that some overlap will occur.

■■● Glomerular Disorders

The majority of the disorders associated with the glomerulus are of immune origin, resulting from immunologic disorders throughout the body, including the kidney. *Immune complexes* formed as a result of immunologic reactions and increased serum immunoglobulins, such as immunoglobulin A (IgA), circulate in the bloodstream and are deposited on the glomerular membranes. Components of the immune system, including complement, neutrophils, lymphocytes, monocytes, and cytokines, are then attracted to the area, producing changes and damage to the membranes. Depending on the immune system mediators involved, damage may consist of cellular infiltration or proliferation resulting in thickening of the glomerular basement membrane, and complement-mediated damage to the capillaries and basement membrane.

Nonimmunologic causes of glomerular damage include exposure to chemicals and toxins that also affect the tubules, disruption of the electrical membrane charges as occurs in the *nephrotic syndrome*, deposition of amyloid material from systemic disorders that may involve chronic inflammation and acute-phase reactants, and the basement membrane thickening associated with diabetic nephropathy.

■■● Glomerulonephritis

The term *glomerulonephritis* refers to a sterile, inflammatory process that affects the glomerulus and is associated with the finding of blood, protein, and casts in the urine. A variety of types of glomerulonephritis exist, and the condition also may progress from one form to another (i.e., rapidly progressive glomerular nephritis (RPGN) to chronic glomerulonephritis to the nephrotic syndrome and eventual renal failure).

Acute Poststreptococcal Glomerulonephritis

As its name implies, acute glomerulonephritis (AGN) is a disease marked by the sudden onset of symptoms consistent with damage to the glomerular membrane. These may include fever; *edema*, most noticeably around the eyes; fatigue; hypertension; oliguria; and hematuria. Symptoms usually occur in children and young adults following respiratory infections caused by cer-

tain strains of group A streptococcus that contain M protein in the cell wall. During the course of the infection, these nephrogenic strains of streptococci form immune complexes with their corresponding circulating antibodies and become deposited on the glomerular membranes. The accompanying inflammatory reaction affects glomerular function.

In most cases, successful management of the secondary complications, hypertension, and electrolyte imbalance, until the immune complexes have been cleared from the blood and the inflammation subsides, results in no permanent kidney damage. Similar symptoms may also be seen following pneumonia, endocarditis, and other severe infections.²

Primary urinalysis findings include marked hematuria, proteinuria, and oliguria, accompanied by red blood cell (RBC) casts, dysmorphic RBCs, hyaline and granular casts, and white blood cells (WBCs). As toxicity to the glomerular membrane subsides, urinalysis results return to normal, with the possible exception of microscopic hematuria that lasts until the membrane damage has been repaired. Blood urea nitrogen (BUN) may be elevated during the acute stages but, like the urinalysis, returns to normal. Demonstration of an elevated serum antistreptolysin O (ASO) titer or anti–group A streptococcal enzyme tests provide evidence that the disease is of streptococcal origin.

Rapidly Progressive (Crescentic) Glomerulonephritis

A more serious form of acute glomerular disease is called rapidly progressive (or crescentic) glomerulonephritis (RPGN) and has a much poorer prognosis, often terminating in renal failure. Symptoms are initiated by deposition of immune complexes in the glomerulus, often as a complication of another form of glomerulonephritis or an immune systemic disorder such as *systemic lupus erythematosus* (SLE). Damage by macrophages to the capillary walls releases cells and plasma into Bowman's space, and the production of crescentic formations containing macrophages, fibroblasts, and polymerized fibrin, causes permanent damage to the capillary tufts.

Initial laboratory results are similar to acute glomerulonephritis but become more abnormal as the disease progresses, including markedly elevated protein levels and very low glomerular filtration rates. Some forms may demonstrate increased fibrin degradation products, cryoglobulins, and the deposition of IgA immune complexes in the glomerulus.³

Goodpasture Syndrome

Morphologic changes to the glomeruli resembling those in RPGN are seen in conjunction with the autoimmune disorder termed Goodpasture syndrome. Appearance of a cytotoxic autoantibody against the glomerular and alveolar basement membranes can follow viral respiratory infections. Attachment of this autoantibody to the basement membrane, followed by complement activation, produces the capillary destruction. Referred to as *antiglomerular basement membrane antibody*, the autoantibody can be detected in patient serum.

Initial pulmonary complaints are *hemoptysis* and *dyspnea*, followed by the development of hematuria. Urinalysis results include proteinuria, hematuria, and the presence of RBC casts. Progression to chronic glomerulonephritis and end-stage renal failure is common.

Wegener's Granulomatosis

Wegener's granulomatosis causes a *granuloma*-producing inflammation of the small blood vessels of primarily the kidney and respiratory system. Key to the diagnosis of Wegener's granulomatosis is the demonstration of antineutrophilic cytoplasmic antibody (ANCA) in the patient's serum.⁴ Binding of these autoantibodies to the neutrophils located in the vascular walls may initiate the immune response and the resulting granuloma formation. Patients usually present first with pulmonary symptoms and later develop renal involvement, including hematuria, proteinuria, RBC casts, and elevated serum creatinine and BUN.

Henoch-Schönlein Pupura

Henoch-Schönlein *purpura* is a disease occurring primarily in children following upper respiratory infections. As its name implies, initial symptoms include the appearance of raised, red patches on the skin. Respiratory and gastrointestinal symptoms, including blood in the sputum and stools, may be present. Renal involvement is the most serious complication of the disorder and may range from mild to heavy proteinuria and hematuria with RBC casts. Complete recovery with normal renal function is seen in more than 50% of patients. In other patients, progression to a more serious form of glomerulonephritis and renal failure may occur. Urinalysis and renal function assessment should be used to monitor patients following recovery from the original symptoms.

Membranous Glomerulonephritis

The predominant characteristic of membranous glomerulonephritis is a pronounced thickening of the glomerular basement membrane resulting from the deposition of immunoglobulin *G* immune complexes. Disorders associated with the development of membranous glomerulonephritis include systemic lupus erythematosus, *Sjögren syndrome*, *secondary* syphilis, hepatitis B, gold and mercury treatments, and malignancy. Many cases of unknown etiology have been reported. As a rule, the disease progresses slowly, with possible remission; however, frequent development of nephrotic syndrome symptoms occurs. There may also be a tendency toward *thrombosis*.

Laboratory findings include microscopic hematuria and elevated urine protein excretion that may reach concentrations similar to those in the nephrotic syndrome. Demonstration of one of the secondary disorders through blood tests can aid in the diagnosis.

Membranoproliferative Glomerulonephritis

Membranoproliferative glomerulonephritis (MPGN) is marked by two different alterations in the cellularity of the glomerulus and peripheral capillaries. Type 1 displays increased cellularity in the subendothelial cells of the mesangium (interstitial area of Bowman's capsule), causing thickening of the capillary walls, whereas type 2 displays extremely dense deposits in the glomerular basement membrane. Many of the patients are children, and the disease has a poor prognosis, with type 1 patients progressing to the nephrotic syndrome and type 2 patients experiencing symptoms of chronic glomerulonephritis. The laboratory findings are variable; however, hematuria, proteinuria, and decreased serum complement levels are usual findings. There appears to be an association with autoimmune disorders, infections, and malignancies.⁶

Chronic Glomerulonephritis



Depending on the amount and duration of the damage occurring to the glomerulus in the previously discussed glomerular disorders, progression to chronic glomerulonephritis and end-stage renal dis-

ease may occur. Gradually worsening symptoms include fatigue, anemia, hypertension, edema, and oliguria.

Examination of the urine reveals hematuria, proteinuria, glucosuria as a result of tubular dysfunction, and many varieties of casts, including broad casts. A markedly decreased glomerular filtration rate is present in conjunction with increased BUN and creatinine levels and electrolyte imbalance.

Immunogloblin A Nephropathy

Also known as Berger disease, IgA *nephropathy*, in which immune complexes containing IgA are deposited on the glomerular membrane, is the most common cause of glomerulonephritis. Patients have increased serum levels of IgA, which may be a result of a mucosal infection. The disorder is most frequently seen in children and young adults.

Patients usually present with an episode of macroscopic hematuria following an infection or strenuous exercise. Recovery from the macroscopic hematuria is spontaneous; however, asymptomatic microhematuria and elevated serum levels of IgA remain. Except for periodic episodes of macroscopic hematuria, a patient with the disorder may remain essentially asymptomatic for 20 years or more; however, there is a gradual progression to chronic glomerulonephritis and end-stage renal disease.

■■● Nephrotic Syndrome



The nephrotic syndrome is marked by massive proteinuria (greater than 3.5 g/d), low levels of serum albumin, high levels of serum lipids, and pronounced edema.¹ Acute onset of the disorder can

146 CHAPTER 8 • Renal Disease

occur in instances of circulatory disruption producing systemic shock that decrease the pressure and flow of blood to the kidney. Progression to the nephrotic syndrome may also occur as a complication of the previously discussed forms of glomerulonephritis.

Increased permeability of the glomerular membrane is attributed to damage to the membrane and changes in the electrical charges in the basal lamina and podocytes, producing a less tightly connected barrier. This facilitates the passage of high-molecular-weight proteins and lipids into the urine. Albumin is the primary protein depleted from the circulation. The ensuing hypoalbuminemia appears to stimulate the increased production of lipids by the liver. The lower oncotic pressure in the capillaries resulting from the depletion of plasma albumin increases the loss of fluid into the interstitial spaces, which, accompanied by sodium retention, produces the edema. Depletion of immunoglobulins and coagulation factors places patients at an increased risk of infection and coagulation disorders. Tubular damage, in addition to glomerular damage, occurs, and the nephrotic syndrome may progress to chronic renal failure.

Urinalysis observations include marked proteinuria; urinary fat droplets; oval fat bodies; renal tubular epithelial (RTE) cells; epithelial, fatty, and waxy casts; and microscopic hematuria. Absorption of the lipid-containing proteins by the RTE cells followed by cellular sloughing produces the characteristic oval fat bodies seen in the sediment examination.

Minimal Change Disease

As the name implies, minimal change disease (also known as lipid nephrosis) produces little cellular change in the glomerulus, although the podocytes appear to be less tightly fitting, allowing for the increased filtration of protein. Patients are usually children who present with edema, heavy proteinuria, transient hematuria, and normal BUN and creatinine results. Although the etiology is unknown at this time, allergic reactions, recent immunization, and possession of the human leukocyte antigen-B12 (HLA-B12) antigen have been associated. The disorder responds well to corticosteroids, and prognosis is generally good, with frequent complete remissions.⁸

■ ■ ● Focal Segmental Glomerulosclerosis

In contrast to the previously discussed disorders, focal segmental *glomerulosclerosis* (FSGS) affects only certain numbers and areas of glomeruli, and the others remain normal. Symptoms may be similar to the nephrotic syndrome and minimal change disease owing to damaged podocytes. Immune deposits, primarily immunoglobulins M and C3, are a frequent finding and can be seen in undamaged glomeruli. FSGS is often seen in association with abuse of heroin and analgesics and with AIDS. Moderate to heavy proteinuria and microscopic hematuria are the most consistent urinalysis findings.

Laboratory testing and clinical information for the glomerular disorders are summarized in Tables 8–1 and 8–2.

Table 8-1 Summary of L	aboratory Testing in Glo	merular Disorders
Disorder	Primary Urinalysis Result	Other Significant Tests
Acute glomerulonephritis	Macroscopic hematuria Proteinuria RBC casts Granular casts	Antistreptolysin O titer Anti–group A streptococcal enzymes
Rapidly progressive glomerulonephritis	Macroscopic hematuria Proteinuria RBC casts	BUN Creatinine Creatinine clearance
Goodpasture syndrome	Macroscopic hematuria Proteinuria RBC casts	Antiglomerular basement membrane antibody
Wegener's granulomatosis	Macroscopic hematuria Proteinuria RBC casts	Antineutrophilic cytoplasmic antibody
Henoch-Schönlein purpura	Macroscopic hematuria Proteinuria RBC casts	Stool occult blood
Membranous glomerulonephritis	Microscopic hematuria Proteinuria	Antinuclear antibody Hepatitis B surface antigen Fluorescent treponemal antibody- absorption test (FTA-ABS)

Disorder	Primary Urinalysis Result	Other Significant Tests
Membranoproliferative glomerulonephritis	Hematuria Proteinuria	Serum complement levels
Chronic glomerulonephritis	Hematuria Proteinuria Glucosuria Cellular and granular casts Waxy and broad casts	BUN Serum creatinine Creatinine clearance Electrolytes
IgA nephropathy (early stages)	Macroscopic or microscopic hematuria	Serum IgA
IgA nephropathy (late stages)	See Chronic glomerulonephritis	
Nephrotic syndrome	Heavy proteinuria Microscopic hematuria Renal tubular cells Oval fat bodies Fat droplets Fatty and waxy casts	Serum albumin Cholesterol Triglycerides
Minimal change disease	Heavy proteinuria Transient hematuria Fat droplets	Serum albumin Cholesterol Triglycerides
Focal segmental glomerulosclerosis	Proteinuria Microscopic hematuria Macroscopic or Microscopic hematuria	Drugs of abuse HIV tests Genetic testing
Alport syndrome (early stages) (late stages) Diabetic nephropathy (late stages)	See Nephrotic syndrome Microalbuminuria See Chronic glomerulonephritis	Blood glucose

Alport Syndrome

Alport syndrome is an inherited disorder affecting the glomerular basement membrane. The syndrome can be inherited as a sex-linked or autosomal genetic disorder. Males are frequently more severely affected than females. During respiratory infections, males before the age of six may exhibit macroscopic hematuria and continue to exhibit microscopic hematuria. Abnormalities in hearing and vision also may be present.

The glomerular basement membrane has a lamellated appearance with areas of thinning. No evidence of glomerular antibodies is present. The prognosis ranges from mild symptoms to persistent hematuria and renal insufficiency in later life to the nephrotic syndrome and end-stage renal disease.

Diabetic Nephropathy

Diabetic nephropathy, also known as Kimmelstiel-Wilson disease, is currently the most common cause of end-stage renal disease. Damage to the glomerular membrane occurs not only

as a result of glomerular membrane thickening but also because of the increased proliferation of mesangial cells and increased deposition of cellular and noncellular material within the glomerular matrix resulting in accumulation of solid substances around the capillary tufts. This is believed to be associated with deposition of glycosylated proteins resulting from poorly controlled blood glucose levels. The vascular structure of the glomerulus also develops sclerosis.

As discussed in Chapter 5, early monitoring of persons diagnosed with diabetes mellitus for the presence of microal-buminuria is important to detect the onset of diabetic nephropathy. Modification of diet and strict control of hypertension can decrease the progression of the renal disease.

■■● Tubular Disorders

Disorders affecting the renal tubules include those in which tubular function is disrupted as a result of actual damage to the tubules and those in which a metabolic or hereditary disorder affects the intricate functions of the tubules.

W. 18 - 10	ith Glomerular Disorders	
Disorder	Etiology	Clinical Course
Acute glomeru- lonephritis	Deposition of immune complexes, formed in conjunction with group A <i>Streptococcus</i> infection, on the glomerular membranes	Rapid onset of hematuria and edema Permanent renal damage seldom occurs
Rapidly progres- sive glomeru- lonephritis	Deposition of immune complexes from systemic immune disorders on the glomerular membrane	Rapid onset with glomerular damage and possible progression to end-stage renal failure
Goodpasture syndrome	Attachment of a cytotoxic antibody formed during viral respiratory infections to glomerular and alveolar basement membranes	Hemoptysis and dyspnea followed by hematuria Possible progression to end-stage renal failure
Wegener's granulomatosis	Antineutrophilic cytoplasmic auto-antibody binds to neutrophils in vascular walls producing damage to small vessels in the lungs and glomerulus	Pulmonary symptoms including hemoptysis develop first followed by renal involvement and possible progression to endstage renal failure
Henoch-Schönlein purpura	Occurs primarily in children following viral respiratory infections; a decrease in platelets disrupts vascular integrity	Initial appearance of purpura followed by blood in sputum and stools and eventual renal involvement Complete recovery is common, but may progress to renal failure
Membranous glomerulonephritis	Thickening of the glomerular membrane following IgG immune complex deposition associated with systemic disorders	Slow progression to the nephrotic syndrome or possible remission
Membranopro- liferative glomeru- lonephritis	Cellular proliferation affecting the capillary walls or the glomerular basement membrane, possibly immune-mediated	Slow progression to chronic glomerulonephritis or nephrotic syndrome
Chronic glomeru- lonephritis	Marked decrease in renal function resulting from glomerular damage precipitated by other renal disorders	Noticeable decrease in renal function progressing to renal failure
IgA nephropathy	Deposition of IgA on the glomerular membrane resulting from increased levels of serum IgA	Recurrent macroscopic hematuria follow- ing exercise with slow progression to chronic glomerulonephritis
Nephrotic syndrome	Disruption of the electrical charges that produce the tightly fitting podocyte barrier resulting in massive loss of protein and lipids	Acute onset following systemic shock Gradual progression from other glomerular disorders and then to renal failure
Minimal change disease	Disruption of the podocytes occuring primarily in children following allergic reactions and immunizations	Frequent complete remission following corticosteroid treatment
Focal segmental glomerulosclerosis	Disruption of podocytes in certain areas of glomeruli associated with heroin and analgesic abuse and AIDS	May resemble nephrotic syndrome or minimal change disease
Alport Syndrome	Genetic disorder showing lamellated and thin- ning of glomerular basement membrane	Slow progression to nephrotic syndrome and end-stage renal disease

Acute Tubular Necrosis



The primary disorder associated with damage to the renal tubules is *acute tubular necrosis* (ATN). Damage to the RTE cells may be produced by decreased blood flow that causes a lack of oxygen

presentation to the tubules (*ischemia*) or the presence of toxic substances in the urinary filtrate.

Disorders causing ischemic ATN include shock, trauma (such as crushing injuries), and surgical procedures. Shock is a general term indicating a severe condition that decreases the flow of blood throughout the body. Examples of conditions that may cause shock are cardiac failures, sepsis involving toxogenic bacteria, anaphylaxis, massive hemorrhage, and contact with high-voltage electricity.

Exposure to a variety of nephrotoxic agents can damage and affect the function of the RTE cells. Substances include the aminoglycoside antibiotics, the antifungal agent amphotericin B, cyclosporine, radiographic dye, organic solvents such as ethylene glycol, heavy metals, and toxic mushrooms. As discussed in Chapter 5, filtration of large amounts of hemoglobin and *myoglobin* is also nephrotoxic.

The disease course of ATN is variable. It may present as an acute complication of an ischemic event or more gradually during exposure to toxic agents. Correction of the ischemia and removal of toxic substances followed by effective management of the accompanying symptoms of acute renal failure frequently result in a complete recovery.

Urinalysis findings include mild proteinuria, microscopic hematuria, and most noticeably the presence of RTE cells and RTE cell casts containing tubular fragments consisting of three or more cells. As a result of the tubular damage, a variety of other casts may be present, including hyaline, granular, waxy, and broad.

Hereditary and Metabolic Tubular Disorders

Disorders affecting tubular function may be caused by systemic conditions that affect or override the tubular reabsorptive maximum (Tm) for particular substances normally reabsorbed by the tubules or by failure to inherit a gene or genes required for tubular reabsorption.

Fanconi Syndrome

The disorder most frequently associated with tubular dysfunction is Fanconi syndrome. The syndrome consists of a generalized failure of tubular reabsorption in the proximal convoluted tubule. Therefore, substances most noticeably affected include glucose, amino acids, phosphorous, sodium, potassium, bicarbonate, and water. Tubular reabsorption may be affected by dysfunction of the transport of filtered substances across the tubular membranes, disruption of cellular energy needed for transport or changes in the tubular membrane permeability.

Fanconi syndrome may be inherited in association with cystinosis and Hartnup disease (see Chapter 9) or acquired

through exposure to toxic agents, including heavy metals and outdated tetracycline, or as a complication of *multiple myeloma* and renal transplant.

Urinalysis findings include glycosuria and possible mild proteinuria.

Nephrogenic Diabetes Insipidus

As discussed in Chapter 2, urine concentration is regulated in the distal convoluted tublules and the collecting ducts in response to antidiuretic hormone (ADH) produced by the hypothalmus. When the action of ADH is disrupted either by the inability of the renal tubules to respond to ADH (nephrogenic DI) or the failure of the hypothalmus to produce ADH (neurogenic DI) excessive amounts of urine are excreted. Differentiation between the two types of DI is covered in Chapter 2.

Nephrogenic diabetes insipidus can be inherited as a sexlinked recessive gene or accquired from medications including lithium and amphotericin B. It also may be seen as a complication of polycystic kidney disease and sickle cell anemia.

Urinalysis findings associated with DI are low specific gravity, pale yellow color, and possible false-negative results for chemical tests.

Renal Glycosuria

In contrast to Fanconi syndrome that exhibits a generalized failure to reabsorb substances from the glomerular filtrate, renal glucosuria affects only the reabsorption of glucose. The disorder is inherited as an autosomal recessive trait. Patients demonstrate increased urine glucose concentrations with normal blood glucose concentrations.

As discussed in Chapter 5, the maximal tubular reabsorption capacity for glucose (Tm_G) is a blood level of approximately 160 to 180 mg/dL. When the Tm_G is reached, glucosuria occurs. In inherited renal glucosuria either the number of glucose transporters in the tubules is decreased or the affinity of the transporters for glucose is decreased.

Laboratory testing and clinical information for the hereditary and metabolic disorders are summarized in Tables 8–3 and 8–4.

Interstitial Disorders

Considering the close proximity between the renal tubules and the renal interstitium, disorders affecting the interstitium also affect the tubules, resulting in the commonly used term *tubulointerstitial disease*. The majority of these disorders involve infections and inflammatory conditions.

The most common renal disease is UTI. Infection may involve the lower urinary tract (urethra and bladder) or the upper urinary tract (renal pelvis, tubules, and interstitium). Most frequently encountered is infection of the bladder (*cystitis*), which if untreated can progress to a more serious upper UTI. Cystitis is seen more often in women and children who present with symptoms of urinary frequency and burning.

Table 8-3 Summary of Laboratory Testing in Metabolic and Tubular Disorders						
Disorder	Primary Urinalysis Results	Other Significant Tests				
Acute tubular necrosis	Microscopic hematuria Proteinuria Renal tubular epithelial cells Renal tubular epithelial cell casts Hyaline, granular, waxy, broad casts	Hemoglobin Hematocrit Cardiac enzymes				
Fanconi syndrome	Glucosuria Possible cystine crystals	Serum and urine electrolytes Amino acid chromatography				
Nephrogenic diabetes insipidus	Low specific gravity Polyuria	ADH testing				
Renal glucosuria	Glucosuria	Blood glucose				

Urinalysis reveals the presence of numerous WBCs and bacteria, often accompanied by mild proteinuria and hematuria and an increased pH.

■ ■ ● Acute Pyelonephritis

Infection of the upper urinary tract, including both the tubules and interstitium, is termed *pyelonephritis* and can occur in both acute and chronic forms. Acute pyelonephritis most frequently occurs as a

result of ascending movement of bacteria from a lower UTI into the renal tubules and interstitium. The ascending movement of bacteria from the bladder is enhanced with conditions that interfere with the downward flow of urine from the ureters to the bladder or the complete emptying of the bladder during urination. These include obstructions such as renal calculi, pregnancy, and reflux of urine from the bladder back into the ureters (visicoureteral reflux). With appropriate antibiotic therapy and removal of any underlying conditions, acute pyelonephritis can be resolved without permanent damage to the tubules.

Patients present with rapid onset of symptoms of urinary frequency and burning and lower back pain. A relatively high correlation between acute pylonephritis and bacteremia has been demonstrated, suggesting the need to perform blood cultures in addition to urine cultures.⁹

Urinalysis results are similar to those seen in cystitis, including numerous leukocytes and bacteria with mild proteinuria and hematuria. The additional finding of WBC casts, signifying infection within the tubules, is of primary diagnostic value for both acute and chronic pyelonephritis. The presence of WBC casts is significant for the differentiation between cystitis and pyelonephritis. Sediments also should be carefully observed for the presence of bacterial casts.

■■● Chronic Pyelonephritis

As its name implies, chronic pyelonephritis is a more serious disorder that can result in permanent damage to the renal tubules and possible progression to chronic renal failure. Congenital urinary structural defects producing a reflux nephropathy are the most frequent cause of chronic pyelonephritis. The

Table 8–4 Summary of Clinical Information Associated With Metabolic and Tubular Disorders				
Disorder	Etiology	Clinical Course		
Acute tubular necrosis	Damage to the renal tubular cells caused by ischemia or toxic agents	Acute onset of renal dysfunction usually resolved when the underlying cause is corrected		
Fanconi syndrome	Inherited in association with cystinosis and Hartnup disease or acquired through exposure to toxic agents	Generalized defect in renal tubular reabsorption requiring supportive therapy		
Nephrogenic diabetes insipidus	Inherited defect of tubular response to ADH or acquired from medications	Requires supportive therapy to prevent dehydration		
Renal glucosuria	Inherited autosomal recessive trait	Benign disorder		

structural abnormalities may cause reflux between the bladder and ureters or within the renal pelvis, affecting emptying of the collecting ducts. Owing to its congenital origin, chronic pyelonephritis is often diagnosed in children and may not be suspected until tubular damage has become advanced.

Urinalysis results are similar to those seen in acute pyelonephritis, particularly in the early stages. As the disease progresses, a variety of granular, waxy, and broad casts accompanied by increased proteinuria and hematuria are present, and renal concentration is decreased.

■ ■ ● Acute Interstitial Nephritis



Acute interstitial nephritis (AIN) is marked by inflammation of the renal interstitium followed by inflammation of the renal tubules. Patients present with a rapid onset of symptoms relating to renal

dysfunction, including oliguria, edema, decreased renal concentrating ability, and a possible decrease in the glomerular filtration rate. Fever and the presence of a skin rash are frequent initial symptoms.

AIN is primarily associated with an allergic reaction to medications that occurs within the renal interstitium, possibly caused by binding of the medication to the interstitial protein. Symptoms tend to develop approximately 2 weeks following

administration of medication. Medications commonly associated with AIN include penicillin, methicillin, ampicillin, cephalosporins, sulfonamides, NSAIDs, and thiazide diuretics. Discontinuation of the offending medication and administration of steroids to control the inflammation frequently results in a return to normal renal function. However, supportive renal dialysis may be required to maintain patients until the inflammation subsides.

Urinalysis results include hematuria, possibly macroscopic, mild to moderate proteinuria, numerous WBCs, and WBC casts without the presence of bacteria. Performing differential leukocyte staining for the presence of increased eosinophils may be useful to confirm the diagnosis. ¹⁰

Laboratory testing and clinical information for the interstitial disorders are summarized in Tables 8–5 and 8–6.

■■● Renal Failure



Renal failure exists in both acute and chronic forms. As discussed in conjunction with many of the previous disorders, this may be a gradual progression from the original disorder to chronic renal failure or

end-stage renal disease. The progression to end-stage renal disease is characterized by a marked decrease in the glomerular filtration rate (less than 25 mL/min); steadily rising serum

Table 8-5 Summary of Laboratory Results in Interstial Disorders					
Disorder	Primary Urinalysis Results	Other Significant Tests			
Cystitis	Leukocyturia Bacteriuria Microscopic hematuria Mild proteinuria Increased pH	Urine culture			
Acute pyelonephritis	Leukocyturia Bacteriuria WBC casts Bacterial casts Microscopic hematuria Proteinuria	Urine culture Blood cultures			
Chronic pyelonephritis	Leukocyturia Bacteriuria WBC casts Bacterial casts Granular, waxy, broad casts Hematuria Proteinuria	Urine culture Blood cultures BUN Creatinine Creatinine clearance			
Acute interstitial nephritis	Hematuria Proteinuria Leukocyturia WBC casts	Urine eosinophils BUN Creatinine Creatinine clearance			

152 CHAPTER 8 • Renal Disease

	ary of Clinical Information Associat nterstitial Disorders	ed
Disorder	Etiology	Clinical Course
Cystitis	Ascending bacterial infection of the bladder	Acute onset of urinary frequency and burning resolved with antibiotics
Acute pyelonephritis	Infection of the renal tubules and interstitium related to interference of urine flow to the bladder, reflux of urine from the bladder, and untreated cystitis	Acute onset of urinary frequency, burning, and lower back pain resolved with antibiotics
Chronic pyelonephritis	Recurrent infection of the renal tubules and interstitium caused by structural abnormalities affecting the flow of urine	Frequently diagnosed in children; requires correction of the underlying structural defect Possible progression to renal failure
Acute interstitial nephritis	Allergic inflammation of the renal interstitium in response to certain medications	Acute onset of renal dysfunction often accompanied by a skin rash Resolves following discontinuation of medication and treatment with corticosteroids

BUN and creatinine values (*azotemia*); electrolyte imbalance; lack of renal concentrating ability producing an isothenuric urine; proteinuria; renal glycosuria; and an abundance of granular, waxy, and broad casts, often referred to as a telescoped urine sediment.

Acute renal failure (ARF), in contrast to chronic renal failure, exhibits a sudden loss of renal function and is frequently reversible. Primary causes of ARF include a sudden

Table 8–7

Causes of Acute Renal Failure

Prerenal

Decreased blood pressure/cardiac output

Hemorrhage

Burns

Surgery

Septicemia

Renal

Acute glomerulonephritis

Acute tubular necrosis

Acute pyelonephritis

Acute interstitial nephritis

Postrenal

Renal calculi

Tumors

Crystallization of ingested substances

decrease in blood flow to the kidney (prerenal), acute glomerular and tubular disease (renal), and renal calculi or tumor obstructions (postrenal). As can be seen from the variety of causes (Table 8–7), patients may present with many different symptoms relating to the particular disorder involved; however, a decreased glomerular filtration rate, oliguria, edema, and azotemia are general characteristics.

Similar to clinical symptoms, urinalysis findings are varied; however, because they relate to the primary cause of the ARF, they can be diagnostically valuable. For example, the presence of RTE cells and casts suggests ATN of prerenal origin; RBCs indicate glomerular injury; WBC casts with or without bacteria indicate interstitial infection or inflammation of renal origin; and postrenal obstruction may show normal and abnormal appearing urothelial cells possibly associated with malignancy.

■ ■ ■ Renal Lithiasis

Renal calculi (kidney stones) may form in the calyces and pelvis of the kidney, ureters, and bladder. In renal *lithiasis*, the calculi vary in size from barely visible to large, staghorn calculi resembling the shape of the renal pelvis and smooth, round bladder stones with diameters of 2 or more inches. Small calculi may be passed in the urine, subjecting the patient to severe pain radiating from the lower back to the legs. Larger stones cannot be passed and may not be detected until patients develop symptoms of urinary obstruction. *Lithotripsy*, a procedure using high-energy shock waves, can be used to break stones located in the upper urinary tract into pieces that can then be passed in the urine. Surgical removal also can be employed.

Conditions favoring the formation of renal calculi are similar to those favoring formation of urinary crystals, including pH, chemical concentration, and urinary stasis. Numerous correlation studies between the presence of crystalluria and the formation of renal calculi have been conducted with varying results. The finding of clumps of crystals in freshly voided urine suggests that conditions may be right for calculus formation. However, owing to the difference in conditions that affect the urine within the body and in a specimen container, little importance can be placed on the role of crystals in the prediction of calculi formation. Increased crystalluria has been noted during the summer months in persons known to form renal calculi. 11

Analysis of the chemical composition of renal calculi plays an important role in patient management. Analysis can be performed chemically, but examination using x-ray crystallography provides a more comprehensive analysis. Approximately 75% of the renal calculi are composed of calcium oxalate or phosphate. Magnesium ammonium phosphate (stuvite), uric acid, and cystine are the other primary calculi constituents. Calcium calculi are frequently associated with metabolic calcium and phosphate disorders and occasionally diet. Magnesium ammonium phosphate calculi are frequently accompanied by urinary infections involving urea-splitting bacteria. The urine pH is often higher than 7.0. Uric acid calculi may be associated with increased intake of foods with high purine content. The urine pH is acidic. Most cystine calculi are seen in conjunction with hereditary disorders of cystine metabolism (see Chapter 9). Patient management techniques include maintaining the urine at a pH incompatible with crystallization of the particular chemicals, maintaining adequate hydration to lower chemical concentration, and suggesting possible dietary restrictions.

Urine specimens from patients suspected of passing or being in the process of passing renal calculi are frequently received in the laboratory. The presence of microscopic hematuria resulting from irritation to the tissues by the moving calculus is the primary urinalysis finding.

References

- Forland, M (ed): Nephrology. Medical Examination Publishing, New York, 1983.
- Johnson, RJ: Nonpoststreptococcal postinfectious glomerulonephritis. In Jacobson, HR, et al: Principles and Practice of Nephrology. BC Decker, Philadelphia, 1991.
- Couser, WG: Rapidly progressive glomerulonephritis. In Jacobson, HR, et al: Principles and Practice of Nephrology. BC Decker, Philadelphia, 1991.
- Kallenberg, CG, Mulder, AH, and Tervaert, JW: Antineutrophil cytoplasmic autoantibodies: A still-growing class of autoantibodies in inflammatory disorders. Am J Med 93(6):675-682, 1992.
- Wasserstein, AG: Membranous glomerulonephritis. In Jacobson, HR, et al: Principles and Practice of Nephrology. BC Decker, Philadelphia, 1991.
- Donadio, JV: Membranoproliferative glomerulonephritis. In Jacobson, HR, et al: Principles and Practice of Nephrology. BC Decker, Philadelphia, 1991.
- 7. Bricker, NS, and Kirschenbaum, MA: The Kidney: Diagnosis and Management. John Wiley, New York, 1984.

- 8. Sherbotle, JR, and Hayes, JR: Idiopathic nephrotic syndrome: Minimal change disease and focal segmental glomerulosclerosis. In Jacobson, HR, et al: Principles and Practice of Nephrology. BC Decker, Philadelphia, 1991.
- 9. Smith, WR, et al: Bacteremia in young urban women admitted with pyelonephritis. Am J Med Sci 313(1):50-57, 1997.
- Bennett, WM, Elzinga, LW, and Porter, GA: Tubulointerstitial disease and toxic nephropathy. In Brenner, BM, and Rector, FC: The Kidney: Physiology and Pathophysiology. WB Saunders, Philadelphia, 1991.
- 11. Hallson, PC, and Rose, GA: Seasonal variations in urinary crystals. Br J Urol 49(4):277-284, 1977.

QUESTIONS

- 1. The majority of glomerular disorders are caused by:
 - A. Sudden drops in blood pressure
 - B. Immunologic disorders
 - C. Exposure to toxic substances
 - D. Bacterial infections
- **2.** Dysmorphic RBC casts would be a significant finding with all of the following *except*:
 - A. Goodpasture syndrome
 - B. Acute glomeruonephritis
 - C. Chronic pyelonephritis
 - D. Henoch-Schönlein purpura
- **3.** Occassional episodes of macroscopic hematuria over periods of 20 or more years are seen with:
 - A. Crescentic glomerulonephritis
 - B. IgA nephropathy
 - C. Nephrotic syndrome
 - D. Wegener's granulomatosis
- **4.** Antiglomerular basement membrane antibody is seen with:
 - A. Wegener's granulomatosis
 - B. IgA nephropathy
 - C. Goodpasture syndrome
 - D. Diabetic nephropathy
- **5.** Antineutrophilic cytoplasmic antibody is diagnostic for:
 - A. IgA nephropathy
 - B. Wegener's granulomatosis
 - C. Henoch-Schönlein purpura
 - D. Goodpasture syndrome
- **6.** Respiratory and renal symptoms are associated with all of the following *except*:
 - A. IgA nephropathy
 - B. Wegener's granulomatosis
 - C. Henoch-Schönlein purpura
 - D. Goodpasture syndrome
- 7. Broad and waxy casts are most frequently seen with:
 - A. Membranoproliferative glomerulonephritis
 - B. Membranous glomerulonephritis
 - C. Chronic glomerulonephritis
 - D. Rapidly progressive glomerulonephritis

154 CHAPTER 8 • Renal Disease

Continued

- **8.** The presence of fatty casts is associated with all of the following *except*:
 - A. Nephrotic syndrome
 - B. Focal segmental glomerulosclerosis
 - C. Nephrogenic diabetes insipidus
 - D. Minimal change disease
- 9. High levels of proteinuria are early symptoms of:
 - A. Alport syndrome
 - B. Diabetic nephropathy
 - C. IgA nephropathy
 - D. Nephrotic syndrome
- 10. Ischemia frequently produces:
- A. Acute renal tubular necrosis
 - B. Minimal change disorder
 - C. Acute renal failure
 - D. Both A and C.
- **11.** A disorder associated with polyuria and low specific gravity is:
 - A. Renal glucosuria
 - B. Cystitis
 - C. Nephrogenic diabetes insipidus
 - D. Focal segmental glomerulosclerosis
- **12**. An inherited or accquired disorder producing a generalized defect in tubular reabsorption is:
 - A. Alport syndrome
 - B. Acute interstitial nephritis
 - C. Fanconi syndrome
 - D. Renal glucosuria
- **13**. The presence of renal tubular epithelial cells and casts is an indication of:
 - A. Acute interstitial nephritis
 - B. Chronic glomerulonephritis
 - C. Minimal change disease
 - D. Acute tubular necrosis
- **14.** Differentiation between cystitis and pyelonephritis is aided by the presence of:
 - A. WBC casts
 - B. RBC casts
 - C. Bacteria
 - D. Granular casts
- **15**. The presence of WBCs and WBC casts with no bacteria seen is indicative of:
 - A. Chronic pyelonephritis
 - B. Acute tubular necrosis
 - C. Acute interstitial nephritis
 - D. Both B and C
- **16**. End-stage renal disease is characterized by all of the following *except*:
 - A. Hypersthenuria
 - B. Isosthenuria
 - C. Azotemia
 - D. Electrolyte imbalance

- 17. Broad and waxy casts are most likely associated with:
 - A. Nephrotic syndrome
 - B. Chronic renal failure
 - C. Focal segmental glomerulosclerosis
 - D. Acute renal failure
- 18. Postrenal acute renal failure could be caused by:
 - A. Ischemia
 - B. Acute tubular necrosis
 - C. Acute interstitial nephritis
 - D. Malignant tumors
- 19. The most common composition of renal calculi is:
 - A. Calcium oxalate
 - B. Magnesium ammonium phosphate
 - C. Cystine
 - D. Uric acid
- **20**. Urinalysis on a patient being evaluated for renal calculi would be most beneficial if it showed:
 - A. Heavy proteinuria
 - B. Calcium oxalate crystals
 - C. Macroscopic hematuria
 - D. Microscopic hematuria

Case Studies and Clinical Situations

1. A 14-year-old boy who has recently recovered from a sore throat develops edema and hematuria. Significant laboratory results include a BUN of 30 mg/dL (normal 8 to 23 mg/dL) and a positive Streptozyme test. Results of a urinalysis are as follows:

COLOR: Red
CLARITY: Cloudy
SP. GRAVITY: 1.020
BILIRUBIN: Negative
UROBILINOGEN: Normal
PROTEIN: 3+
GLUCOSE: Negative

LEUKOCYTE: Trace

Microscopic:

100 RBCs/hpf—many dysmorphic forms

- 5-8 WBCs/hpf
- 0-2 granular casts/lpf
- 0-1 RBC casts/lpf
- a. What disorder do these results and history indicate?
- b. What specific characteristic was present in the organism causing the sore throat?
- c. What is the significance of the dysmorphic RBCs?
- d. Are the WBCs significant? Why or why not?
- e. What is the expected prognosis of this patient?
- f. If the above urinalysis results were seen in a 5year-old boy who has developed a red, patchy rash following recovery from a sore throat, what disorder would be suspected?

2. B.J. is a seriously ill 40-year-old man with a history of several episodes of macroscopic hematuria in the past 20 years. The episodes were associated with exercise or stress. Until recently the macroscopic hematuria had spontaneously reverted to asymptomatic microscopic hematuria. Significant laboratory results include a BUN of 80 mg/dL (normal 8 to 23 mg/dL), serum creatinine of 4.5 mg/dL (normal 0.6 to 1.2 mg/dL), creatinine clearance of 20 mL/min (normal 107 to 139 mL/min), serum calcium of 8.0 mg/dL (normal 9.2 to 11.0 mg/dL), serum phosphorus of 6.0 mg/dL (normal 2.3 to 4.7 mg/dL), and an elevated level of serum IgA. Results of a routine urinalysis are as follows:

COLOR: Red KETONES: Negative CLARITY: Slightly cloudy BLOOD: Large SP. GRAVITY: 1.010 BILIRUBIN: Negative UROBILINOGEN: Normal рн: 6.5 PROTEIN: 300 mg/dL NTRITE: Negative GLUCOSE: 250 mg/dL LEUKOCYTE: Trace

Microscopic:

>100 RBCs/hpf 2-4 hyaline casts/lpf 8-10 WBCs/hpf 1-5 granular casts/lpf 0-2 waxy casts/lpf 0-2 broad waxy

- a. What specific disease do the patient's laboratory results and history suggest?
- b. Which laboratory result is most helpful in diagnosing this disease?
- c. What additional diagnosis does his current condition suggest?
- d. What is the significance of the positive result for urine glucose?
- e. Is the specific gravity significant? Why or why not?
- f. What is the significance of the waxy casts?
- 3. A 45-year-old woman recovering from injuries received in an automobile accident that resulted in her being taken to the emergency room with severe hypotension develops massive edema. Significant laboratory results include a BUN of 30 mg/dL (normal 8 to 23 mg/dL), cholesterol of 400 mg/dL (normal 150 to 240 mg/dL), triglycerides of 840 mg/dL (normal 10 to 190 mg/dL), serum protein of 4.5 mg/dL (normal 6.0 to 7.8 mg/dL), albumin of 2.0 mg/dL (normal 3.2 to 4.5 mg/dL), and a total urine protein of 3.8 g/d (normal 100 mg/d). Urinalysis results are as follows:

COLOR: Yellow KETONES: Negative CLARITY: Cloudy BLOOD: Moderate SP. GRAVITY: 1.015 BILIRUBIN: Negative рн: 6.0 UROBILINOGEN: Normal PROTEIN: 4+ NITRITE: Negative GLUCOSE: Negative LEUKOCYTE: Negative

Microscopic:

15–20 RBCs/hpf 0–2 granular casts/lpf Moderate free fat droplets

0–5 WBCs/hpf 0–2 fatty casts/lpf Moderate cholesterol crystals

0–2 oval fat bodies/hpf

- a. What renal disorder do these results suggest?
- b. How does the patient's history relate to this disorder?
- c. What physiologic mechanism accounts for the massive proteinuria?
- d. What is the relationship of the proteinuria to the edema?
- e. What mechanism produces the oval fat bodies?
- f. State two additional procedures that could be performed to verify the presence of the oval fat bodies and the fatty casts.
- 4. A routinely active 4-year-old boy becomes increasingly less active after receiving several preschool immunizations. His pediatrician observes noticeable puffiness around the eyes. A blood test shows normal BUN and creatinine results and markedly decreased total protein and albumin values. Urinalysis results are as follows:

COLOR: Yellow KETONES: Negative CLARITY: Hazy BLOOD: Small SP. GRAVITY: 1.018 BILIRUBIN: Negative UROBILINOGEN: Normal рн: 6.5 PROTEIN: 4+ NITRITE: Negative GLUCOSE: Negative LEUKOCYTE: Negative Microscopic:

10-15 RBCs/hpf 0-1 hyaline casts/lpf 0-4 WBCs/hpf 0-2 granular casts/lpf Moderate fat droplets 0-1 oval fat bodies/hpf

- a. What disorder do the patient history, physical appearance, and laboratory results suggest?
- b. What other renal disorder produces similar urinalysis results?
- c. What is the expected prognosis for this patient?
- 5. A 32-year-old construction worker experiences respiratory difficulty followed by the appearance of bloodstreaked sputum. He delays visiting a physician until symptoms of extreme fatigue and red urine are present. A chest radiograph shows pulmonary infiltration and sputum culture is negative for pathogens. Blood test results indicate anemia, increased BUN and creatinine, and the presence of antiglomerular basement membrane antibody. Urinalysis results are as follows:

COLOR: Red KETONES: Negative CLARITY: Cloudy BLOOD: Large SP. GRAVITY: 1.015 BILIRUBIN: Negative UROBILINOGEN: Normal рн: 6.0 NITRITE: Negative PROTEIN: 3+ GLUCOSE: Negative LEUKOCYTE: Trace

156 CHAPTER 8 • Renal Disease

Continued

Microscopic:

100 RBCs/hpf 0–3 hyaline casts/lpf 10–15 WBCs/hpf 0–3 granular casts/lpf 0–2 RBCs casts/lpf

- a. What disorder do the laboratory results suggest?
- b. How is this disorder affecting the glomerulus?
- c. If the antiglomerular membrane antibody test was negative, what disorder might be considered?
- d. What is the diagnostic test for this disorder?
- e. By what mechanism does this disorder affect the glomerulus?
- 6. A 25-year-old pregnant woman comes to the outpatient clinic with symptoms of lower back pain, urinary frequency, and a burning sensation when voiding. Her pregnancy has been normal up to this time. She is given a sterile container and asked to collect a midstream clean-catch urine specimen. Routine urinalysis results are as follows:

COLOR: Pale yellow
CLARITY: Hazy
SP. GRAVITY: 1.005
BILIRUBIN: Negative
UROBILINOGEN: Normal
PROTEIN: Trace
GLUCOSE: Negative
LEUKOCYTE: 2+

Microscopic:

6–10 RBCs/hpf Heavy bacteria 40–50 WBCs/hpf Moderate squamous epithelial cells

- a. What is the most probable diagnosis for this patient?
- b. What is the correlation between the color and the specific gravity?
- c. What is the significance of the blood and protein tests?
- d. Is this specimen suitable for the appearance of glitter cells? Explain your answer.
- e. What other population is at a high risk for developing this condition?
- f. What disorder might develop if this disorder is not treated?
- **7.** A 10-year-old patient with a history of recurrent UTIs is admitted to the hospital for diagnostic tests. Initial urinalysis results are as follows:

COLOR: Yellow KETONE: Negative
CLARITY: Cloudy BLOOD: Small
SP. GRAVITY: 1.025 BILIRUBIN: Negative
ph: 8.0 UROBILINOGEN: Normal
PROTEIN: 2+ NITRITE: Positive

(SSA): (2+)

GLUCOSE: Negative LEUKOCYTE: 2+

Microscopic:

6–10 RBCs/hpf 0–2 WBC casts/lpf Many bacteria >100 WBCs/hpf 0–1 bacterial casts/lpf with clumps

A repeat urinalysis a day later has the following

results:

COLOR: Yellow KETONES: Negative CLARITY: Cloudy BLOOD: Small SP. GRAVITY: >1.035 BILIRUBIN: Negative UROBILINOGEN: Normal PROTEIN: 2+ NITRITE: Positive

(SSA): (4+)

GLUCOSE: Negative LEUKOCYTE: 2+

Microscopic:

6–10 RBCs/hpf 0–2 WBC casts/lpf Many bacteria >100 WBCs/hpf 0–1 bacterial casts/lpf Moderate birefringent, flat crystals

- a. What diagnostic procedure was performed on the patient that could account for the differences in the two urinalysis results?
- b. Considering the patient's age and history, what is the most probable diagnosis?
- c. What microscopic constituent is most helpful to this diagnosis?
- d. What is the most probable cause of this disorder?
- e. How can the presence of the bacterial cast be confirmed?
- f. What is the most probable source of the crystals present in the sediment?
- g. Without surgical intervention, what is the patient's prognosis?
- **8.** A 35-year-old patient being treated for a sinus infection with methicillin develops fever, a skin rash, and edema. Urinalysis results are as follows:

COLOR: Dark yellow
CLARITY: Cloudy
SP. GRAVITY: 1.012
BILIRUBIN: Negative
ph: 6.0
PROTEIN: 3+
GLUCOSE: Negative

KETONES: Negative
BLOOD: Moderate
BILIRUBIN: Negative
UROBILINOGEN: Normal
NITRITE: Negative
LEUKOCYTE: 2+

Microscopic:

20–30 RBCs/hpf 1–2 WBC casts/lpf >100 WBCs/hpf 1–2 granular casts/lpf After receiving the urinalysis report, the physician orders a test for urinary eosinophils. The urinary eosinophil result is 10%.

- a. Is the urinary eosinophil result normal or abnormal?
- b. What is the probable diagnosis for this patient?
- c. Discuss the significance of the increased WBCs and WBC casts in the absence of bacteria.
- d. How can this condition be corrected?
- 9. Following surgery to correct a massive hemorrhage, a 55-year-old patient exhibits oliguria and edema. Blood test results indicate increasing azotemia and electrolyte imbalance. The glomerular filtration rate is 20 mL/min. Urinalysis results are as follows:

COLOR: Yellow KETONES: Negative BLOOD: Moderate

SP. GRAVITY: 1.010

ph: 7.0

PROTEIN: 3+

GLUCOSE: 2+

Microscopic:

BILIRUBIN: Negative

UROBILINOGEN: Normal

NITRITE: Negative

LEUKOCYTE: Negative

Microscopic:

50–60 RBCs/hpf 2–3 granular casts/lpf 3–6 WBCs/hpf 2–3 RTE cell casts/lpf 3–4 RTE cells/hpf 0–1 waxy casts/lpf

0-1 broad granular casts/lpf

- a. What diagnosis do the patient's history and laboratory results suggest?
- b. What is the most probable cause of the patient's disorder? Is this considered to be of prerenal, renal, or postrenal origin?
- c. What is the significance of the specific gravity result?
- d. What is the significance of the RTE cells?
- e. State two possible reasons for the presence of the broad casts.
- 10. A 40-year-old man develops severe back and abdominal pain after dinner. The pain subsides during the night but recurs in the morning, and he visits his family physician. Results of a complete blood count and an amylase are normal. Results of a routine urinalysis are as follows:

COLOR: Dark yellow
CLARITY: Hazy
SP. GRAVITY: 1.030
BILIRUBIN: Negative
PROTEIN: Trace
GLUCOSE: Negative

KETONES: Negative
BLOOD: Moderate
BL

Microscopic

15-20 RBCs/hpf-appear crenated

0-2 WBCs/hpf

Few squamous epithelial cells

- a. What condition could these urinalysis results and the patient's symptoms represent?
- b. What would account for the crenated RBCs?
- c. Is there a correlation between the urine color and specific gravity and the patient's symptoms?
- d. Based on the primary substance that causes this condition, what type of crystals might have been present?
- e. What changes will the patient be advised to make in his lifestyle to prevent future occurrences?
- **11**. State a disorder or disorders that relate to each of the following descriptions:
 - a. A patient with severe lower back pain and microscopic hematuria is scheduled for lithotripsy.
 - b. The patient exhibits pulmonary and renal symptoms and a positive ANCA test.
 - A patient who tested positive for HIV exhibits mild symptoms resembling the nephrotic syndrome.
 - d. A 40-year-old patient diagnosed with SLE develops macroscopic hematuria, proteinuria, and the presence of RBC casts in the urine sediment.
 - e. A 50-year-old patient diagnosed with SLE exhibits symptoms of gradually declining renal function and increasing proteinuria.
 - f. A patient who has taken outdated tetracycline develops glycosuria and a generalized aminoaciduria.
 - g. A patient known to form renal calculi develops oliguria, edema, and azotemia.
 - h. A patient has a normal blood glucose and glucosuria. State two disorders.
 - i. A 6-year-old boy with respiratory syncytial virus has macroscopic hematuria.











Urine Screening for Metabolic Disorders

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

- Explain the abnormal accumulation of metabolites in the urine in terms of overflow and renal disorders.
- **2** Discuss the importance and procedures for newborn screening.
- 3 Name the metabolic defect in phenylketonuria, and describe the clinical manifestations it produces
- 4 Discuss the performance of the tandem mass spectrophotometry and ferric chloride tests and their roles in the detection and management of phenylketonuria.
- 5 State three causes of tyrosyluria and the screening tests for its presence.
- 6 Name the abnormal urinary substance present in alkaptonuria, and tell how its presence may be suspected.
- 7 Discuss the appearance and significance of urine that contains melanin.
- **8** Describe a basic laboratory observation that has relevance in maple syrup urine disease.
- **9** Discuss the significance of ketonuria in a newborn.
- 10 Differentiate between the presence of urinary indican owing to intestinal disorders and Hartnup disease.

- 11 State the significance of increased urinary 5-hydroxyindoleacetic acid.
- 12 Differentiate between cystinuria and cystinosis, including the differences that are found during analysis of the urine and the disease processes.
- 13 Name the chemical screening test for cystine.
- Describe the components in the heme synthesis pathway, including the primary specimens used for their analysis.
- 15 Briefly discuss the major porphyrias with regard to cause and clinical significance.
- Differentiate between the Ehrlich reaction and fluorescent testing with regard to the testing of porphyrin components.
- **17** Describe the appearance of urine that contains increased porphyrins.
- Define mucopolysaccharides, and name three syndromes in which they are involved.
- **19** List three screening tests for the detection of urinary mucopolysaccharides.
- **20** State the significance of increased uric acid crystals in newborns' urine.
- **21** Explain the reason for performing tests for urinary-reducing substances on all newborns.

KEY TERMS

alkaptonuria
cystinosis
cystinuria
galactosuria
homocystinuria
inborn error of metabolism

indicanuria
Lesch-Nyhan disease
maple syrup urine disease
melanuria
melituria

mucopolysaccharidoses organic acidemias phenylketonuria porphyrinuria tyrosyluria

As has been discussed in previous chapters, many of the abnormal results obtained in the routine urinalysis are related to metabolic rather than renal disease. Urine as an end product of body metabolism may contain additional abnormal substances not tested for by routine urinalysis. Often, these substances can be detected or monitored by additional screening tests that can also be performed in the urinalysis laboratory. Positive screening tests can then be followed up with more sophisticated procedures performed in other sections of the laboratory.

The need to perform additional tests may be detected by the observations of alert laboratory personnel during the performance of the routine analysis or from observations of abnormal specimen color and odor by nursing staff and patients (Table 9–1). In other instances, clinical symptoms and family histories are the deciding factors.

Overflow Versus Renal Disorders

The appearance of abnormal metabolic substances in the urine can be caused by a variety of disorders that can generally be grouped into two categories, termed the overflow type and renal type. Overflow disorders result from the disruption of a normal metabolic pathway that causes increased plasma concentrations of the nonmetabolized substances. These chemicals either override the reabsorption ability of the renal tubules or are not normally reabsorbed from the filtrate because they

are only present in minute amounts. Abnormal accumulations of the renal type are caused by malfunctions in the tubular reabsorption mechanism, as discussed in Chapter 8.

The most frequently encountered abnormalities are associated with metabolic disturbances that produce urinary overflow of substances involved in protein, fat, and carbohydrate metabolism. This is understandable when one considers the vast number of enzymes used in the metabolic pathways of proteins, fats, and carbohydrates and the fact that their function is essential for complete metabolism. Disruption of enzyme function can be caused by failure to inherit the gene to produce a particular enzyme, referred to as an *inborn error of metabolism* (IEM), 1 or by organ malfunction from disease or toxic reactions. The most frequently encountered abnormal urinary metabolites are summarized in Table 9–2, and their appearance is classified according to functional defect. This table also includes substances and conditions that are covered in this chapter.

■ ■ ● Newborn Screening Tests

Traditionally, many of the urine tests discussed in this chapter were performed primarily to detect and monitor newborns for IEMs. In recent years the screening of newborns has increased to include more sensitive detection methods and ever-increasing levels of state-mandated tests for IEMs. Many states currently require testing for as many as 29 metabolic disorders.²

	rmal Metabolic Constitutions Detected in the R	
Color	Odor	Crystals
Homogentisic acid	Phenylketonuria	Cystine
Melanin	Maple syrup urine disease	Leucine
Indican	Isovaleric acidemia	Tyrosine
Porphyrins	Cystinuria Cystinosis Homocystinuria	Lesch-Nyhan disease

Metaboli Metaboli	sorders of Protein and sm Associated With A ents Classified as to Fu	bnormal Urinary		
Overflow Inherited	Metabolic	Renal		
Phenylketonuria	Infantile tyrosinemia	Hartnup disease		
Tyrosinemia	Melanuria	Cystinuria		
Alkaptonuria	Indicanuria			
Maple syrup urine disease	5-Hydroxyindoleacetic acid	5-Hydroxyindoleacetic acid		
Organic acidemias	Porphyria			
Cystinosis				
Porphyria				
Mucopolysaccharidoses				
Galactosemia				
Lesch-Nyhan disease				

As discussed later in this chapter, because many of these disorders cause the buildup of unmetabolized toxic food ingredients, it is important that the defects be detected early in life. Levels of these substances are elevated more rapidly in blood than urine. Therefore, blood collected by infant heel puncture is initially tested. Testing for many substances is now performed using tandem mass spectrophotometry (MS/MS). MS/MS is capable of screening the infant blood sample for specific substances associated with particular IEMs. This is covered in more depth in clinical chemistry classes. Methods for specific gene testing are also rapidly being developed.

Amino Acid Disorders

The amino acid disorders with urinary screening tests include phenylketonuria (PKU), tyrosyluria, alkaptonuria, melanuria, maple syrup urine disease, organic acidemias, indicanuria, cystinuria, and cystinosis.

Phenylalanine-Tyrosine Disorders

Many of the most frequently requested special urinalysis procedures are associated with the phenylalanine-tyrosine metabolic pathway. Major inherited disorders include PKU, tyrosyluria, and alkaptonuria. Metabolic defects cause production of excessive amounts of melanin. The relationship of these varied disorders is illustrated in Figure 9-1.

Phenylketonuria

The most well known of the *aminoacidurias*, PKU is estimated to occur in 1 of every 10,000 to 20,000 births and, if undetected, results in severe mental retardation. It was first identified in Norway by Ivan Følling in 1934, when a mother

with other mentally retarded children reported a peculiar mousy odor to her child's urine. Analysis of the urine showed increased amounts of the keto acids, including phenylpyruvate. As shown in Figure 9-1, this occurs when the normal conversion of phenylalanine to tyrosine is disrupted. Interruption of the pathway also produces children with fair complexions—even in dark-skinned families—owing to the decreased production of tyrosine and its pigmentation metabolite melanin.

PKU is caused by failure to inherit the gene to produce the enzyme phenylalanine hydroxylase. The gene is inherited as an autosomal recessive trait with no noticeable characteristics or defects exhibited by heterozygous carriers. Fortunately, screening tests are available for early detection of the abnormality, and all states have laws that require the screening of newborns for PKU.² Once discovered, dietary changes that eliminate phenylalanine, a major constituent of milk, from the infant's diet can prevent the excessive buildup of serum phenylalanine and can thereby avoid damage to the child's mental capabilities. As the child matures, alternate pathways of phenylalanine metabolism develop, and dietary restrictions can be eased. Many products that contain large amounts of phenylalanine, such as aspartame, now have warnings for people with PKU.

The initial screening for PKU does not come under the auspices of the urinalysis laboratory, because increased blood levels of phenylalanine must, of course, occur prior to the urinary excretion of phenylpyruvic acid, which may take from 2 to 6 weeks. Blood samples must be obtained before the newborn is discharged from the hospital. The increasing tendency to release newborns from the hospital as early as 24 hours after birth has caused concern about the ability to detect increased phenylalanine levels at that early stage. Studies have shown that in many cases phenylalanine can be detected as

162 CHAPTER 9 • Urine Screening for Metabolic Disorders

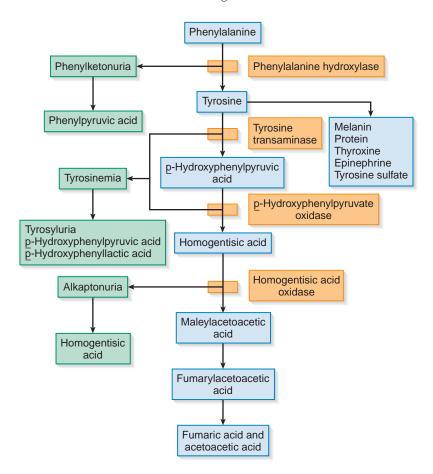


Figure 9–I Phenylalanine and tyrosine metabolism.

early as 4 hours after birth and, if the cutoff level for normal results is lowered from 4 mg/dL to 2 mg/dL, the presence of PKU should be detected. Tests may need to be repeated during an early visit to the pediatrician.³ More girls than boys escape detection of PKU during early tests because of slower rises in blood phenylalanine levels.¹

Urine testing can be used as a follow-up procedure in questionable diagnostic cases, as a screening test to ensure proper dietary control in previously diagnosed cases, and, more recently, as a means of monitoring the dietary intake of pregnant women known to lack phenylalanine hydroxylase.

The most well-known blood test for PKU is the microbial inhibition assay developed by Guthrie.⁴ In this procedure, blood from a heelstick is absorbed into filter paper circles. The circle must be completely saturated with a single layer of blood. The blood-impregnated disks are then placed on culture media streaked with the bacterium Bacillus subtilis. If increased phenylalanine levels are present in the blood, phenylalanine counteracts the action of beta-2-thienylalanine, an inhibitor of B. subtilis that is present in the media, and growth will be observed around the paper disks. Notice that in Figure 9-2 the bacterial growth around the disk from patient A corresponds to the positive control, indicating an increased level of phenylalanine. Modifications of the Guthrie test can also detect other disorders including maple syrup urine disease, homocystinuria, tyrosinemia, histidinemia, valinemia, and galactosemia.5 MS/MS tests for many other

PROCEDURE



Ferric Chloride Tube Test

- 1. Place 1 mL of urine in a tube.
- 2. Slowly add five drops of 10% ferric chloride.
- 3. Observe color.

substances, including thyroid hormones, trypsin, and biotinidase, can also be performed from dried blood collected by heel stick.

Urine tests for phenylpyruvic acid are based upon the ferric chloride reaction performed by tube test. As will be seen in other discussions in this chapter, the ferric chloride test is a nonspecific reaction and will react with many other amino acids and commonly ingested medications (see Table 9–4 later in the chapter). Some brands of disposable diapers

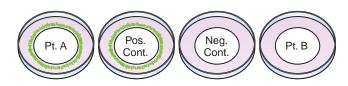


Figure 9-2 Guthrie test.

also produce false-positive reactions for PKU when tested with ferric chloride.⁶ The addition of ferric chloride to urine containing phenylpyruvic acid produces a permanent bluegreen color.

Tyrosyluria



The accumulation of excess tyrosine in the plasma (tyrosinemia) producing urinary overflow may be due to several causes and is not well categorized. As can be seen in Table 9–2, disorders of tyrosine

metabolism may result from either inherited or metabolic defects. Also, because two reactions are directly involved in the metabolism of tyrosine, the urine may contain excess tyrosine or its degradation products, *p*-hydroxyphenylpyruvic acid and *p*-hydroxyphenyllactic acid.

Most frequently seen is a transitory tyrosinemia in premature infants, which is caused by underdevelopment of the liver function required to produce the enzymes necessary to complete the tyrosine metabolism. This condition seldom results in permanent damage, but it may be confused with PKU when urinary screening tests are performed, because the ferric chloride test produces a green color. This reaction can be distinguished from the PKU reaction in the ferric chloride tube test because the green color fades rapidly when tyrosine is present.

Acquired severe liver disease also produces tyrosyluria resembling that of the transitory newborn variety and, of course, is a more serious condition. In both instances, rarely seen tyrosine and leucine crystals may be observed during microscopic examination of the urine sediment.

Hereditary disorders in which enzymes required in the metabolic pathway are not produced present a serious and usually fatal condition that results in both liver and renal tubular disease producing a generalized aminoaciduria. Based on the enzymes affected, the hereditary disorders can be classified into three types all producing tyrosylemia and tyrosyluria. Type 1 is caused by the deficiency of the enzyme fumarylacetoacetate hydrolase (FAH). Type 1 produces a generalized renal tubular disorder and progressive liver failure in infants soon after birth. Type 2 tyrosinemia is caused by lack of the enzyme tyrosine aminotransferase. Persons develop corneal erosion and lesions on the palms, fingers, and soles of the feet believed to be caused by crystallization of tyrosine in the cells. Type 3 tyrosinemia is caused by lack of the enzyme p-hydroxyphenylpyruvic acid dioxygenase. This can result in mental retardation if dietary restrictions of phenylalanine and tyrosine are not implemented.

The urinary screening test for tyrosine and its metabolites is the nitroso-naphthol test. Like the ferric chloride test, the nitroso-naphthol test is nonspecific and, as shown in Table 9–4, reacts with compounds other than tyrosine and its metabolites. However, the presence of an orange-red color shows a positive reaction and indicates that further testing is needed. Screening tests using MS/MS are available for tyrosinemia types 1 and 2.

PROCEDURE



Nitroso-Napthol Test

- 1. Place five drops of urine in a tube.
- 2. Add 1 mL of 2.63N nitric acid.
- 3. Add one drop of 21.5% sodium nitrite.
- 4. Add 0.1 mL 1-nitroso-2-napthol.
- 5 Mix
- 6. Wait 5 minutes.
- 7. Observe color.

Melanuria

The previous discussion has focused on the major phenylalanine-tyrosine metabolic pathway illustrated in Figure 9-1; however, as also shown in Figure 9-1 and is the case with many amino acids, a second metabolic pathway also exists for tyrosine. This pathway is responsible for the production of melanin, thyroxine, epinephrine, protein, and tyrosine-sulfate. Of these substances, the major concern of the urinalysis laboratory is melanin, the pigment responsible for the dark color of hair, skin, and eyes. Deficient production of melanin results in *albinism*.

Increased urinary melanin produces a darkening of urine. The darkening appears after the urine is exposed to air. Elevation of urinary melanin is a serious finding that indicates the overproliferation of the normal melanin-producing cells (melanocytes) producing a malignant melanoma. These tumors secrete a colorless precursor of melanin, 5,6-dihydroxyindole, which oxidizes to melanogen and then to melanin, producing the characteristic dark urine.

Melanin reacts with ferric chloride, sodium nitroprusside (nitroferricyanide), and Ehrlich reagent. In the ferric chloride tube test, a gray or black precipitate forms in the presence of melanin and is easily differentiated from the reactions produced by other amino acid products. The sodium nitroprusside test provides an additional screening test for melanin. A red color is produced by the reaction of melanin and sodium nitroprusside. Interference due to a red color from acetone and creatinine can be avoided by adding glacial acetic acid, which causes melanin to revert to a green-black color, whereas acetone turns purple, and creatinine becomes amber.

PROCEDURE



Homogentisic Acid Test

- 1. Place 4 mL of 3% silver nitrate in a tube.
- 2. Add 0.5 mL of urine.
- **3.** Mix.
- 4. Add 10% NH₄OH by drops.
- 5. Observe for black color.

164

Alkaptonuria

Alkaptonuria was one of the six original inborn errors of metabolism described by Garrod in 1902. The name alkaptonuria was derived from the observation that urine from patients with this condition darkened after becoming alkaline from standing at room temperature. Therefore, the term "alkali lover," or alkaptonuria, was adopted. This metabolic defect is actually the third major one in the phenylalaninetyrosine pathway and occurs from failure to inherit the gene to produce the enzyme homogentisic acid oxidase. Without this enzyme, the phenylalanine-tyrosine pathway cannot proceed to completion, and homogentisic acid accumulates in the blood, tissues, and urine. This condition does not usually manifest itself clinically in early childhood, but observations of brown-stained or black-stained cloth diapers and reddishstained disposable diapers have been reported.⁷ In later life, brown pigment becomes deposited in the body tissues (particularly noticeable in the ears). Deposits in the cartilage eventually lead to arthritis. A high percentage of persons with alkaptonuria develop liver and cardiac disorders.5

Homogentisic acid reacts in several of the routinely used screening tests for metabolic disorders, including the ferric chloride test, in which a transient deep blue color is produced in the tube test. A yellow precipitate is produced in the Clinitest, indicating the presence of a reducing substance. Another screening test for urinary homogentisic acid is to add alkali to freshly voided urine and to observe for darkening of the color; however, large amounts of ascorbic acid interfere with this reaction. The addition of silver nitrate and ammonium hydroxide also produces a black urine. A spectrophotometric method to obtain quantitative measurements of both urine and plasma homogentisic acid is available, as are chromatography procedures. It is important to differentiate between the presence of homogenistic acid and melanin when urine is seen that has turned black upon standing.

Summary of Urine Screening Tests for Disorders of the Phenylalanine-Tyrosine Pathway

Phenylketonuria	Clinitest
Ferric chloride tube test	Alkalization of fresh urine
Tyrosyluria	Melanuria
Ferric chloride tube test	Ferric chloride tube test
Nitroso-naphthol test	Sodium nitroprusside test
Alkaptonuria	Ehrlich test
Ferric chloride tube test	

Branched-Chain Amino Acid Disorders

The branched-chain amino acids differ from other amino acids by having a methyl group that branches from the main aliphatic carbon chain. Two major groups of disorders are associated with errors in the metabolism of the branched-chain amino acids. In one group, accumulation of one or more of the early amino acid degradation products occurs as is seen in maple syrup urine disease. Disorders in the other group are termed organic acidemias and result in accumulation of organic acids produced further down in the amino acid metabolic pathway.

A significant laboratory finding in branched-chain amino acid disorders is the presence of ketonuria in a newborn.

Maple Syrup Urine Disease

Although maple syrup urine disease (MSUD) is rare, a brief discussion is included in this chapter because the urinalysis laboratory can provide valuable information for the essential early detection of this disease.

MSUD is caused by an IEM, inherited as an autosomal recessive trait. The amino acids involved are leucine, isoleucine, and valine. The metabolic pathway begins normally, with the transamination of the three amino acids in the liver to the keto acids (α -ketoisovaleric, α -ketoisocaproic, and α -keto- β -methylvaleric). Failure to inherit the gene for the enzyme necessary to produce oxidative decarboxylation of these keto acids results in their accumulation in the blood and urine. 1

Newborns with MSUD begin to exhibit clinical symptoms associated with failure to thrive after approximately l week. The presence of the disease may be suspected from these clinical symptoms; however, many other conditions have similar symptoms. Personnel in the urinalysis laboratory or in the nursery may detect the disease through the observation of a urine specimen that produces a strong odor resembling maple syrup that is caused by the rapid accumulation of keto acids in the urine. Even though a report of urine odor is not a part of the routine urinalysis, notifying the physician about this unusual finding can prevent the development of severe mental retardation and even death. Studies have shown that if maple syrup urine disease is detected by the 11th day, dietary regulation and careful monitoring of urinary keto acid concentrations can control the disorder.8 MSUD is included in many newborn screening profiles using MS/MS.

The urine screening test most frequently performed for keto acids is the 2,4-dinitrophenylhydrazine (DNPH) reaction. The DNPH test can also be used for home monitoring of diagnosed patients. Adding DNPH to urine that contains keto acids produces a yellow turbidity or precipitate. Large doses of ampicillin interfere with the DNPH reaction. Like many other urinary screening tests, the DNPH reaction is not specific for maple syrup urine disease, inasmuch as keto acids are present in other disorders, including PKU. In addition, all specimens with a positive reagent strip test result for ketones produce a positive DNPH result. However, treatment can be started on the basis of odor, clinical symptoms,

and a positive DNPH test while confirmatory procedures are being performed.

Organic Acidemias

Generalized symptoms of the organic acidemias include early severe illness, often with vomiting accompanied by metabolic acidosis; hypoglycemia; ketonuria; and increased serum ammonia.⁹ The three most frequently encountered disorders are isovaleric, propionic, and methylmalonic acidemia.

Isovaleric acidemia may be suspected when urine specimens, and sometimes even the patient, possess a characteristic odor of "sweaty feet." This is caused by the accumulation of isovalerylglycine due to a deficiency of isovaleryl coenzyme A in the leucine pathway. There is no urine screening test for isovalerylglycine. The presence of isovaleric, propionic, and methylmalonic acidemias can be included in newborn screening programs using MS/MS.

Propionic and methylmalonic acidemias result from errors in the metabolic pathway converting isoleucine, valine, threonine, and methionine to succinyl coenzyme A. Propionic acid is the immediate precursor to methylmalonic acid in this pathway.

A urine test is available for methylmalonic aciduria. The procedure uses p-nitroaniline to produce an emerald green color in the presence of methylmalonic acid.¹⁰

PROCEDURE



2,4-Dinitrophenylhydrazine (DNPH) Test

- 1. Place 1 mL of urine in a tube.
- 2. Add 10 drops of 0.2% 2,4-DNPH in 2N HCl.
- 3. Wait 10 minutes.
- 4. Observe for yellow or white precipitate.

Tryptophan Disorders

The major concern of the urinalysis laboratory in the metabolism of tryptophan is the increased urinary excretion of the metabolites indican and 5-hydroxyindoleacetic acid (5-HIAA). Figure 9-3 shows a simplified diagram of the metabolic pathways by which these substances are produced. Other metabolic pathways of tryptophan are not included because they do not relate directly to the urinalysis laboratory.

Indicanuria

Under normal conditions, most of the tryptophan that enters the intestine is either reabsorbed for use by the body in the production of protein or is converted to indole by the intestinal bacteria and excreted in the feces. However, in certain intestinal disorders (including obstruction; the presence of abnormal bacteria; malabsorption syndromes; and Hartnup disease, a rare inherited disorder) increased amounts of tryptophan are converted to indole. The excess indole is then reabsorbed from the intestine into the bloodstream and circulated to the liver, where it is converted to indican and then excreted in the urine. Indican excreted in the urine is colorless until oxidized to the dye indigo blue by exposure to air. Early diagnosis of Hartnup disease is sometimes made when mothers report a blue staining of their infant's diapers, referred to as the "blue diaper syndrome." Urinary indican reacts with acidic ferric chloride to form a deep blue or violet color that can subsequently be extracted into chloroform.

Except in cases of Hartnup disease, correction of the underlying intestinal disorder returns urinary indican levels to normal. The inherited defect in Hartnup disease affects not only the intestinal reabsorption of tryptophan but also the renal tubular reabsorption of other amino acids, resulting in a generalized aminoaciduria (Fanconi syndrome). The defective renal transport of amino acids does not appear to affect other renal tubular functions. Therefore, with proper dietary sup-

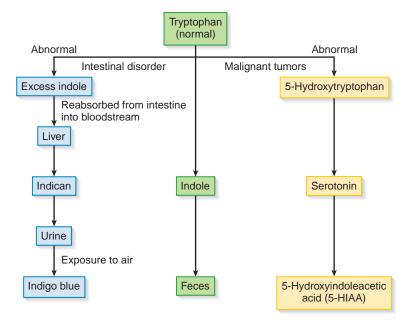


Figure 9–3 Tryptophan metabolism.

PROCEDURE

p-Nitroaniline Test

- 1. Place one drop of urine in a tube.
- 2. Add 15 drops of 0.1% *p*-nitroaniline in 0.16 M HCl.
- 3. Add five drops of 0.5% sodium nitrite.
- **4.** Mix.
- 5. Add 1 mL of 1 M sodium acetate buffer at pH 4.3.
- 6. Boil for 1 minute.
- 7. Add five drops of 8N NaOH.
- 8. Observe for emerald green color.

plements, including niacin, persons with Hartnup disease have a good prognosis. 11

5-Hydroxyindoleacetic Acid

As shown in Figure 9-3, a second metabolic pathway of tryptophan is for the production of serotonin used in the stimulation of smooth muscles. Serotonin is produced from tryptophan by the argentaffin cells in the intestine and is carried through the body primarily by the platelets. Normally, the body uses most of the serotonin, and only small amounts of its degradation product 5-hydroxyindoleacetic acid (HIAA) are available for excretion in the urine. However, when carcinoid tumors involving the argentaffin (enterochromaffin) cells develop, excess amounts of serotonin are produced, resulting in the elevation of urinary 5-HIAA levels.

The addition of nitrous acid and 1-nitroso-2-naphthol to urine that contains 5-HIAA causes the appearance of a purple to black color, depending on the amount of 5-HIAA present. The normal daily excretion of 5-HIAA is 2 to 8 mg, and excretion of greater than 25 mg/24 h can be an indication of argentaffin cell tumors. The test can be performed on a random or first morning specimen; however, false-negative results can occur based on the specimen concentration and also because 5-HIAA may not be produced at a constant rate throughout the day. If a 24-hour sample is used, it must be preserved with hydrochloric or boric acid. Patients must be given explicit dietary instructions prior to the collection of any sample to be tested for 5-HIAA, because serotonin is a major constituent of foods such as bananas, pineapples, and tomatoes. Medications, including phenothiazines and acetanilids, also cause interference. Patients should be requested to withhold medications for 72 hours prior to specimen collection.

Cystine Disorders



There are two distinct disorders of cystine metabolism that exhibit renal manifestations. Confusion as to their relationship existed for many years following the discovery by Wollaston in 1810 of renal cal-

culi consisting of cystine. It is now known that, although both disorders are inherited, one is a defect in the renal tubular

PROCEDURE



Cyanide-Nitroprusside Test

- 1. Place 3 mL of urine in a tube.
- 2. Add 2 mL sodium cyanide.
- 3. Wait 10 minutes.
- 4. Add five drops 5% sodium nitroprusside.
- 5. Observe for red-purple color.

transport of amino acids (cystinuria) and the other is an IEM (cystinosis). A noticeable odor of sulfur may be present in the urine in disorders of cystine metabolism.

Cystinuria

As the name implies, the condition is marked by elevated amounts of the amino acid cystine in the urine. The presence of increased urinary cystine is not due to a defect in the metabolism of cystine but, rather, to the inability of the renal tubules to reabsorb cystine filtered by the glomerulus. The demonstration that not only cystine but also lysine, arginine, and ornithine are not reabsorbed has ruled out the possibility of an error in metabolism even though the condition is inherited. 12 The disorder has two modes of inheritance: one in which reabsorption of all four amino acids—cystine, lysine, arginine, and ornithine—is affected, and the other in which only cystine and lysine are not reabsorbed. Genetic studies have grouped cystinuria into three types based on the two inherited genes and their heterozyous and homozygous inheritance. In general, persons with any form of inheritance may form renal calculi but the calculi are less common in persons in whom only lysine and cystine are affected. 13 Approximately 65% of the people in whom all four amino acids are affected can be expected to produce calculi early in life.

Because cystine is much less soluble than the other three amino acids, laboratory screening determinations are based on the observation of cystine crystals in the sediment of concentrated or first morning specimens. Cystine is also the only amino acid found during the analysis of calculi from these patients. A chemical screening test for urinary cystine can be performed using cyanide-nitroprusside. Reduction of cystine by sodium cyanide followed by the addition of nitroprusside produces a red-purple color in a specimen that contains excess cystine. False-positive reactions occur in the presence of ketones and homocystine, and additional tests may have to be performed.

Cystinosis



Regarded as a genuine IEM, cystinosis can occur in three variations, ranging from a severe fatal disorder developed in infancy to a benign form appearing in adulthood. The disorder has two general categories,

termed nephopathic and nonnephropathic. The nepropathic

category is subdivided into infantile and late-onset cystinosis. A defect in the lysosomal membranes prevents the release of cystine into the cellular cytoplasm for metabolism. The incomplete metabolism of cystine results in crystalline deposits of cystine in many areas of the body, including the cornea, bone marrow, lymph nodes, and internal organs. A major defect in the renal tubular reabsorption mechanism (Fanconi syndrome) also occurs. The renal tubules, particularly the proximal convoluted tubules, are affected by the deposits of cystine that interfere with reabsorption. This is not an inherited disorder of renal tubular reabsorption as seen in cystinuria. Continued deposition of cystine, if untreated, results in renal failure early in life. In infantile nephropathic cystinosis, there is rapid progression to renal failure. In lateonset nephropathic cystinosis there is a gradual progression to total renal failure. Renal transplants and the use of cystinedepleting medications to prevent the buildup of cystine in other tissues are extending lives. Nonnephropathic cystinosis is relatively benign but may cause some ocular disorders.

Routine laboratory findings in infantile nephropathic cystinosis include polyuria, generalized aminoaciduria, positive test results for reducing substances, and lack of urinary concentration.

Homocystinuria

Defects in the metabolism of the amino acid methioine produce an increase in homocystine throughout the body. The increased homocystine can result in failure to thrive, cataracts, mental retardation, thromboembolic problems, and death. Early detection of this disorder and a change in diet that excludes foods high in methionine can alleviate the metabolic problems. Therefore, screening for homocystine is included in most newborn screening programs. Newborn screening tests originally performed using a modification of the Guthrie microbial inhibition assay have been replaced with MS/MS testing.

As mentioned, increased urinary homocystine gives a positive result with the cyanide-nitroprusside test. Therefore, an additional screening test for homocystinuria must be performed by following a positive cyanide-nitroprusside test result with a silver-nitroprusside test, in which only homocystine will react. The use of silver nitrate in place of sodium cyanide reduces homocystine to its nitroprusside-reactive

PROCEDURE



Silver Nitroprusside Test

- 1. Place 1 mL of urine in a tube.
- 2. Add two drops concentrated NH₄OH.
- 3. Add 0.5 mL 5% silver nitrate.
- 4. Wait 10 minutes.
- 5. Add five drops sodium nitroprusside.
- 6. Observe for red-purple color.

form but does not reduce cystine. Consequently, a positive reaction in the silver-nitroprusside test confirms the presence of homocystinuria. ¹⁰ Fresh urine should be used when testing for homocystine.

Porphyrin Disorders

Porphyrins are the intermediate compounds in the production of heme. The basic pathway for heme synthesis presented in Figure 9-4 shows the three primary porphyrins (uroporphyrin, coproporphyrin, and protoporphyrin) and the porphyrin precursors (α -aminolevulinic acid [ALA] and porphobilinogen). As can be seen, the synthesis of heme can be blocked at a number of stages. Blockage of a pathway reaction results in the accumulation of the product formed just prior to the interruption. Detection and identification of this product in the urine, bile, feces, or blood can then aid in determining the cause of a specific disorder.

The solubility of the porphyrin compounds varies with their structure. ALA, porphobilinogen, and uroporphyrin are the most soluble and readily appear in the urine. Coproporphyrin is less soluble but is found in the urine, whereas protoporphyrin is not seen in the urine. Fecal analysis has usually been performed for the detection of coproporphyrin and protoporphyrin. However, to avoid false-positive interference, bile is a more acceptable specimen. ¹⁴ The Centers for Disease Control and Prevention (CDC) recommends analysis of whole blood for the presence of free erythrocyte protoporphyrin (FEP) as a screening test for lead poisoning.

Disorders of porphyrin metabolism are collectively termed *porphyrias*. They can be inherited or acquired from erythrocytic and hepatic malfunctions or exposure to toxic agents. Common causes of acquired porphyrias include lead poisoning, excessive alcohol exposure, iron deficiency, chronic liver disease, and renal disease. Inherited porphyrias are much rarer than acquired porphyrias. They are caused by failure to inherit the gene that produces an enzyme needed in the metabolic pathway. In Figure 9-4, the enzyme deficiency sites for some of the more common porphyrias are shown. The inherited porphyrias are frequently classified by their clinical symptoms, either neurologic/psychiatric or cutaneous photosensitivity or a combination of both (Table 9–3).

An indication of the possible presence of *porphyrinuria* is the observation of a red or port wine color to the urine after exposure to air. The port wine urine color is more prevalent in the erythropoetic porphyrias, and staining of the teeth may also occur. As seen with other inherited disorders, the presence of congenital porphyria is sometimes suspected from a red discoloration of an infant's diapers.

The two screening tests for porphyrinuria use the Ehrlich reaction and fluorescence under ultraviolet light in the 550- to 600-nm range. The Ehrlich reaction can be used only for the detection of ALA and porphobilinogen. Acetylacetone must be added to the specimen to convert the ALA to porphobilinogen prior to performing the Ehrlich test. The fluorescent technique must be used for the other porphyrins. The Ehrlich reaction, including the Watson-Schwartz test for differentia-

HEME SYNTHESIS

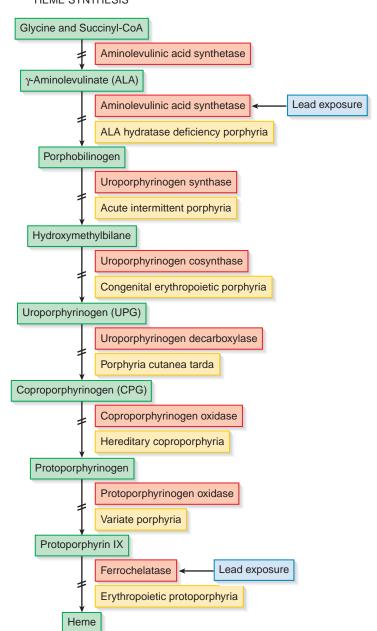


Figure 9–4 Pathway of heme formation, including stages affected by the major disorders of porphyrin metabolism.

tion between the presence of urobilinogen and porphobilinogen and the Hoesch test, were discussed in detail in Chapter 5. Testing for the presence of porphobilinogen is most useful when patients exhibit symptoms of an acute attack. Increased porphobilinogen is associated with acute intermittent porphyria. A negative test result is obtained in the presence of lead poisoning unless ALA is first converted to porphobilinogen.

Fluorescent screening for the other porphyrins uses their extraction into a mixture of glacial acetic acid and ethyl acetate. The solvent layer is then examined. Negative reactions have a faint blue fluorescence. Positive reactions fluoresce as violet, pink, or red, depending on the concentration of porphyrins. If the presence of interfering substances is suspected, the organic layer can be removed to a separate tube, and 0.5 mL of

hydrochloric acid added to the tube. Only porphyrins are extracted into the acid layer, which then produces a bright orange-red fluorescence. The fluorescence method does not distinguish among uroporphyrin, coproporphyrin, and protoporphyrin, but it rules out porphobilinogen and ALA. The identification of the specific porphyrins requires additional techniques and the analysis of fecal and erythrocyte samples. Increased protoporphyrin is best measured in whole blood.

Historical Note

Did you ever wonder why the concept of vampires was started? Think about the previous discussion on the symptoms and inheritance of porphrias.

Table 9-3 Summary of Most Common Porphyrias			
Porphyria	Elevated Compound(s)	Clinical Symptoms	Laboratory Testing
Acute intermittent porphyria	ALA Porphobilinogen	Neurologic/psychiatric	Urine/Ehrlich reaction
Porphyria cutanea tarda	Uroporphyrin	Photosensitivity	Urine fluorescence
Congenital erythropoietic porphyria	Uroporphyrin Coproporphyrin	Photosensitivity	Urine or feces fluorescence
Variegate porphyria	Coproporphyrin	Photosensitivity/ neurologic	Bile or feces fluorescence
Erythropoietic protoporphyria	Protoporphyrin	Photosensitivity	Blood FEP Bile or feces fluorescence
Lead poisoning	ALA	Neurologic	Acetoacetic acid + urine/Ehrlich reaction
	Protoporphyrin		Blood FEP

Photosensitivity Avoidance of sunlight
Pale coloring Anemia caused by heme disorder
Port wine-colored urine/
red-stained teeth
Psychiatric symptoms Abnormal behavior
Inherited disorder Familial association, small gene
pool

Dracula is associated with Transylvania, now Romania. Porphyria was a common disease of early royalty in Europe as a result of intermarriage among the royals of different countries. King George III reportedly died blind, deaf, and mad from porphyria.

Mucopolysaccharide Disorders

Mucopolysaccharides, or glycosaminoglycans, are a group of large compounds located primarily in the connective tissue. They consist of a protein core with numerous polysaccharide branches. Inherited disorders in the metabolism of these compounds prevent the complete breakdown of the polysaccharide portion of the compounds, resulting in accumulation of the incompletely metabolized polysaccharide portions in the lysosomes of the connective tissue cells and their increased

PROCEDURE



Cetytrimethylammonium Bromide (CTAB) Test

- 1. Place 5 mL of urine in a tube.
- 2. Add 1 mL 5% CTAB in citrate buffer.
- 3. Read turbidity in 5 minutes.

PROCEDURE



Mucopolysaccharide (MPS) Paper Test

- 1. Dip filter paper into 0.59% azure A dye in 2% acetic acid.
- 2. Dry.
- 3. Add one drop of urine to paper.
- **4.** Wash with 1 mL acetic acid + 200 mL methanol diluted to a liter.
- 5. Observe for blue color.

excretion in the urine. The products most frequently found in the urine are dermatan sulfate, keratan sulfate, and heparan sulfate, with the appearance of a particular substance being determined by the specific metabolic error that was inherited. ¹⁵ Therefore, identification of the specific enzyme deficiency is necessary to establish a specific diagnosis.

There are many types of *mucopolysaccharidoses*, but the best known are Hurler syndrome, Hunter syndrome, and Sanfilippo syndrome. In both Hurler and Hunter syndromes, the skeletal structure is abnormal and there is severe mental retardation; in Hurler syndrome, mucopolysaccharides accumulate in the cornea of the eye. Hunter syndrome is inherited as sex-linked recessive and is rarely seen in females. Without treatment, both syndromes are usually fatal during childhood, whereas in Sanfilippo syndrome, the only abnormality is mental retardation. Bone marrow transplants and gene replacement therapy are the most promising treatments for these disorders.

Urinary screening tests for mucopolysaccharides are requested either as part of a routine battery of tests performed on all newborns or on infants who exhibit symptoms of mental retardation or failure to thrive. The most frequently used screening tests are the acid-albumin and cetyltrimethylammonium bromide (CTAB) turbidity tests and the metachromatic staining spot tests. In both the acid-albumin and the CTAB tests, a thick, white turbidity forms when these reagents are added to urine that contains mucopolysaccharides. Turbidity is usually graded on a scale of 0 to 4 after 30 minutes with acid-albumin and after 5 minutes with CTAB.¹⁶

Metachromatic staining procedures use basic dyes to react with the acidic mucopolysaccharides. Papers can be prepared by dipping Whatman No. 1 filter paper into a 0.59% azure A dye in 2% acetic acid and letting it air dry. Urine that contains mucopolysaccharides produces a blue spot that cannot be washed away by a dilute acidified methanol solution.

Purine Disorders



A disorder of purine metabolism known as *Lesch-Nyhan disease* that is inherited as a sex-linked recessive results in massive excretion of urinary uric acid crystals. Failure to inherit the gene to produce

the enzyme hypoxanthine guanine phosphoribosyltransferase is responsible for the accumulation of uric acid throughout the body. Patients suffer from severe motor defects, mental retardation, a tendency toward self-destruction, gout, and renal calculi. Development is usually normal for the first 6 to 8 months, with the first symptom often being the observation of uric acid crystals resembling orange sand in diapers. Laboratories should be alert for the presence of increased uric acid crystals in pediatric urine specimens.

Carbohydrate Disorders

The presence of increased urinary sugar (*melituria*) is most frequently due to an inherited disorder. In fact, *pentosuria* was one of Garrod's original six IEMs. ¹⁸ Fortunately, the majority of meliturias cause no disturbance to body metabolism. ¹⁹ However, as discussed in Chapter 5, pediatric urine should be routinely screened for the presence of reducing substances using Clinitest. The finding of a positive copper reduction test result combined with a negative reagent strip glucose oxidase test result is strongly suggestive of a disorder

PROCEDURE



Fructose Screening Test

- 1. Place 5 mL of urine in a tube.
- 2. Add 5 mL of 25% HCl.
- 3. Boil 5 minutes.
- 4. Add 5 mg resorcinol.
- 5. Boil 10 seconds.
- 6. Observe for a red precipitate.

of carbohydrate metabolism. Of primary concern is the presence of *galactosuria*, indicating the inability to properly metabolize galactose to glucose. The resulting galactosemia with toxic intermediate metabolic products results in infant failure to thrive, combined with liver disorders, cataracts, and severe mental retardation. Early detection of galactosuria followed by removal of lactose (a disaccharide containing galactose and glucose) from the diet can prevent these symptoms.

Galactosuria can be caused by a deficiency in any of three enzymes, galactose-1-phosphate uridyl transferase (GALT), galactokinase and UDP-galactose-4-epimerase. Of these enzymes, it is GALT deficiency that causes the severe, possible fatal symptoms associated with galactosemia. Newborn screening programs currently test for the presence of GALT deficiency. The enzyme is measured in the red blood cells as part of the newborn heel puncture protocol. As a result, persons with deficiencies in the other two enzymes may still produce glactosuria but have negative newborn screening tests. Galactose kinase deficiency can result in cataracts in adulthood. UDP-galactose-4-epimerase deficiency may be asymptomatic or produce mild symptoms.

Other causes of melituria include lactose, fructose, and pentose. *Lactosuria* may be seen during pregnancy and lactation. *Fructosuria* is associated with parenteral feeding and pentosuria with ingestion of large amounts of fruit. Additional tests including chromatography can be used to identify other nonglucose reducing substances.

Urine screening tests for metabolic disorders discussed in this chapter are summarized in Table 9–4.

Table 9–4	Comparison of Urinary Screening Tests		
Test	Disorder	Observation	
Color	Alkaptonuria	Black	
	Melanuria	Black	
	Indicanuria	Dark blue	
	Porphyrinuria	Port wine	
Odor	Phenylketonuria	Mousy	
	Maple syrup urine disease	Maple syrup	
	Isovaleric acidemia	Sweaty feet	

Test	Disorder	Observation
	Cystinuria Cystinosis Homocystinuria	Sulphur Sulphur Sulphur
Crystals	Tyrosyluria Cystinuria Lesch-Nyhan disease	Sheaths of fine needles Colorless hexagonal plates Yellow-brown crystals
Ferric chloride tube test	Phenylketonuria Tyrosyluria Alkaptonuria Melanuria Maple syrup urine disease Indicanuria 5-HIAA	Blue-green Transient green Transient blue Gray-black Green-gray Violet-blue with chloroform Blue-green
Nitroso-naphthol	Tyrosyluria Maple syrup urine disease 5-HIAA	Red Red Violet with nitric acid
2,4-Dinitrophenylhydrazine	Phenylketonuria Tyrosyluria Maple syrup urine disease Isovaleric acidemia Propionic acidemia Methylmalonic acidemia	Yellow Yellow Yellow Yellow Yellow
Acetest	Maple syrup urine disease Isovaleric acidemia Propionic acidemia Methylmalonic acidemia Melanuria	Purple Purple Purple Purple Red
<i>p</i> -Nitroaniline	Methylmalonic acidemia	Emerald green
Cyanide-nitroprusside	Cystinuria Cystinosis Homocystinuria	Red-purple Red-purple Red-purple
Silver nitroprusside	Homocystinuria Alkaptonuria	Red-purple Black
Ehrlich reaction	Porphyrinuria Melanuria	Red Red
Cetytrimethylammonium bromide	Mucopolysaccharidoses	White turbidity
Mucopolysaccharide paper	Mucopolysaccharidoses	Blue spot
Clinitest	Melituria Cystinosis Alkaptonuria	Orange-red Orange-red Orange-red

172

References

- 1. Frimpton, GW: Aminoacidurias due to inherited disorders of metabolism. N Engl J Med 1289:835-901, 1973.
- National newborn screening and genetics resource center. Available at: http:// www.genes-r-us.uthscsa.edu Accessed January 17, 2007.
- 3. Doherty, LB, Rohr, FJ, and Levy, HL: Detection of phenylketonuria in the very early newborn specimen. Pediatrics 87(2):240-244, 1991.
- 4. Guthrie, R: Blood screening for phenylketonuria. JAMA 178(8):863, 1961.
- Stanbury, JB: The Metabolic Basis of Inherited Diseases. McGraw-Hill, New York, 1983.
- Kishel, M, and Lighty, P: Some diaper brands give false-positive tests for PKU. N Engl J Med 300(4):200, 1979.
- 7. Nyhan, WL, and Sakati, NO: Diagnostic Recognition of Genetic Disease. Lea & Febiger, Philadelphia, 1987.
- 8. Clow, CL, Reade, TH, and Scriver, CR: Outcome of early and long-term management of classical maple syrup urine disease. Pediatrics 68(6):856-862, 1981.
- 9. Goodman, SI: Disorders of organic acid metabolism. In Emery, AEH, and Rimoin, DL: Principles and Practice of Medical Genetics. Churchill Livingstone, New York, 1990.
- Thomas, GH, and Howell, RR: Selected Screening Tests for Metabolic Diseases. Yearbook Medical Publishers, Chicago, 1973.
- 11. Jepson, JB: Hartnup's disease. In Stanbury, JB, Wyngaarden, JB, and Fredrickson, DS (eds): The Metabolic Basis of Inherited Diseases. McGraw-Hill, New York, 1983.
- 12. Nyhan, WL: Abnormalities in Amino Acid Metabolism in Clinical Medicine. Appleton-Century-Crofts, Norwalk, Conn., 1984.
- 13. Dello Strolongo, L, et al: Comparison between SLC3A1 and SLC7A9 cysinuria patients and carriers: A need for a new classification. J Am Soc Nephrol 13:2547-2553, 2002.
- 14. Nuttall, KL: Porphyrins and disorders of porphyrin metabolism. In Burtis, CA, and Ashwood, ER: Tietz Fundamentals of Clinical Chemistry. WB Saunders, Philadelphia, 1996.
- 15. McKusick, VA, and Neufeld, EF: The mucopolysaccharide storage diseases. In Stanbury, JB, Wyngaarden, JB, and Fredrickson, DS (eds): The Metabolic Basis of Inherited Diseases. McGraw-Hill, New York, 1983.
- 16. Kelly, S: Biochemical Methods in Medical Genetics. Charles C. Thomas, Springfield, Ill., 1977.
- 17. Bordon, M: Screening for metabolic disease. In Nyhan, WL: Abnormalities in Amino Acid Metabolism in Clinical Medicine. Appleton-Century-Crofts, Norwalk, Conn., 1984.
- 18. Garrod, AE: Inborn Errors of Metabolism. Henry Froude & Hodder & Stoughton, London, 1923.
- 19. Hiatt, HH: Pentosuria. In Stanbury, JB, Wyngaarden, JB, and Fredrickson, DS (eds): The Metabolic Basis of Inherited Diseases. McGraw-Hill, New York, 1983.

QUESTIONS STUDY

- 1. All states require newborn screening for PKU for early:
 - A. Modifications of diet
 - B. Administration of antibiotics
 - C. Detection of diabetes
 - D. Initiation of gene therapy

- **2.** All of the following disorders can be detected by newborn screening *except*:
 - A. Tyrosyluria
 - B. MSUD
 - C. Melanuria
 - D. Galactosemia
- 3. The best specimen for early newborn screening is a:
 - A. Timed urine specimen
 - B. Blood specimen
 - C. First morining urine specimen
 - D. Fecal specimen
- **4.** Abnormal urine screening tests categorized as an overflow disorder include all of the following *except*:
 - A. Alkaptonuria
 - B. Galactosemia
 - C. Melanuria
 - D. Cystinuria
- **5.** Which of the following disorders is not associated with the phenylalanine-tyrosine pathway?
 - A. MSUD
 - B. Alkaptonuria
 - C. Albinism
 - D. Tyrosinemia
- 6. Urine screening tests for PKU utilize:
 - A. Microbial inhibition
 - B. Nitroso-napthol
 - C. Dinitrophenylhydrazine
 - D. Ferric chloride
- 7. The least serious form of tyrosylemia is:
 - A. Immature liver function
 - B. Type 1
 - C. Type 2
 - D. Type 3
- **8.** An overflow disorder of the phenylalanine-tyrosine pathway that could produce a false-positive reaction with the reagent strip test for ketones is:
 - A. Alkaptonuria
 - B. Melanuria
 - C. MSUD
 - D. Tyrosyluria
- **9.** An overflow disorder that could produce a false-positive reaction with clinitest is:
 - A. Cystinuria
 - B. Alkaptonuria
 - C. Indicanuria
 - D. Porphyrinuria
- **10.** A urine that turns black after sitting by the sink for several hours could be indicative of:
 - A. Alkaptonuria
 - B. MSUD
 - C. Melanuria
 - D. Both A and C

11. Ketonuria in a newborn is an indication of: A. MSUD
B. Isovaleric acidemia
C. Methylmalonic acidemia
D. All of the above
12. Urine from a newborn with MSUD will have a
significant:
A. Pale color
B. Yellow precipitate
C. Milky appearance D. Sweet odor
13. A substance that reacts with p-nitroaniline is: A. Isovaleric acid
B. Propionic acid
C. Methylmalonic acid
D. Indican
14. Which of the following has a significant odor?
A. Isovaleric acidemia
B. Propionic acidemia
C. Methylmalonic acidemia
D. Indicanuria
15. Hartnup disease is a disorder associated with the
metabolism of:
A. Organic acids
B. Tryptophan
C. Cystine
D. Phenylalanine
16. 5-HIAA is a degradation product of:
A. Heme B. Indole
C. Serotonin
D. Melanin
17. Elevated urinary levels of 5-HIAA are associated with:
A. Carcinoid tumors
B. Hartnup disease
C. Cystinuria
D. Platelet disorders
18. False-positive levels of 5-HIAA can be caused by
a diet high in:
A. Meat
B. Carbohydrates
C. Starch D. Bananas
19. Place the appropriate letter in front of the following statements.
A. Cystinuria
B. Cystinosis
IEM
Inherited disorder of renal tubules
Fanconi syndrome
Cystine deposits in the cornea
Early renal calculi formation

- **20.** Urine from patients with cystine disorders will react with:
 - A. Dinitrophenylhydrazine
 - B. Sodium cyanide
 - C. Ehrlich reagent
 - D. 1-Nitroso-napthol
- **21**. Blue diaper syndrome is associated with:
 - A. Lesch-Nyhan syndrome
 - B. Phenylketonuria
 - C. Cystinuria
 - D. Hartnup disease
- **22**. Homocystinuria is caused by failure to metabolize:
 - A. Lysine
 - B. Methionine
 - C. Arginine
 - D. Cystine
- 23. Early detection is most valuable for correction of:
 - A. Homocystinuria
 - B. Cystinuria
 - C. Indicanuria
 - D. Porphyrinuria
- **24**. The Ehrlich reaction will only detect the presence of:
 - A. Aminolevulinic acid
 - B. Porphobilinogen
 - C. Coproporphyrin
 - D. Protoporphyrin
- **25.** Acetylacetone is added to the urine prior to performing the Ehrlich test when checking for:
 - A. Aminolevulinic acid
 - B. Porphobilinogen
 - C. Uroporphyrin
 - D. Coproporphyrin
- **26**. The classic urine color associated with porphyria is:
 - A. Dark yellow
 - B. Indigo blue
 - C. Pink
 - D. Port wine
- **27**. Which of the following specimens can be used for porphyrin testing?
 - A. Urine
 - B. Blood
 - C. Feces
 - D. All of the above
- **28.** The two stages of heme formation affected by lead poisoning are:
 - A. Porphobilinogen and uroporphyrin
 - B. Aminolevulinic acid and porphobilinogen
 - C. Coproporphyrin and protoporphyrin
 - D. Aminolevulinic acid and protoporphyrin

Continued

- **29**. Hurler, Hunter, and Sanfilippo syndromes are hereditary disorders affecting metabolism of:
 - A. Porphyrins
 - B. Purines
 - C. Mucopolysaccharides
 - D. Tryptophan
- **30.** Many uric acid crystals in a pediatric urine specimen may indicate:
 - A. Hurler syndrome
 - B. Lesch-Nyhan disease
 - C. Melituria
 - D. Sanfilippo syndrome
- 31. Deficiency of the GALT enzyme will produce a:
 - A. Positive Clinitest
 - B. Glycosuria
 - C. Galactosemia
 - D. Both A and C
- **32.** Match the metabolic urine disorders with their classic urine abonormalities.

____PKU A. Sulfur odor
____Indicanuria B. Sweaty feet odor
____Cystinuria C. Orange sand in diaper
____Homogentisic acid D. Mousy odor

____Lesch-Nyhan disease

Case Studies and Clinical Situations

E. Black color

F. Blue color

- 1. A premature infant develops jaundice. Laboratory tests are negative for hemolytic disease of the newborn, but the infant's bilirubin level continues to rise. Abnormal urinalysis results include a dark yellow color, positive Ictotest, and needle-shaped crystals seen on microscopic examination.
 - a. What is the most probable cause of the infant's jaundice?
 - b. How will urine from this infant react in the ferric chloride test?
 - c. Could these same urine findings be associated with an adult? Explain your answer.
 - d. What kind of crystals are present? Name another type of crystal with a spherical shape that is associated with this condition.
 - e. When blood is drawn from this infant, what precaution should be taken to ensure the integrity of the specimen?

- 2. A newborn develops severe vomiting and symptoms of metabolic acidosis. Urinalysis results are positive for ketones and negative for glucose and other reducing substances.
 - a. State a urinalysis screening test that would be positive in this patient.
 - b. If the urine had an odor of "sweaty feet," what metabolic disorder would be suspected?
 - c. If the newborn was producing dark brown urine with a sweet odor, what disorder would be suspected?
 - d. State an additional urinalysis screening test that might be ordered on the infant. If this test produces an emerald green color, what is the significance?
 - e. The urine produces a green-gray color when tested with ferric chloride. Is this an expected result? Why or why not?
 - f. For the most accurate diagnosis of the newborn's condition, what additional testing should be performed?
- **3.** A 13-year-old boy is awakened with severe back and abdominal pain and is taken to the emergency room by his parents. A complete blood count is normal. Family history shows that both his father and uncle are chronic kidney stone formers. Results of a urinalysis are as follows:

COLOR: Yellow
APPEARANCE: Hazy
SP. GRAVITY: 1.025
BILIRUBIN: Negative
PROTEIN: Negative
GLUCOSE: Negative

KETONES: Negative
BLOOD: Moderate
BLIRUBIN: Negative
UROBILINOGEN: Normal
NITRITE: Negative
LEUKOCYTE: Negative

Microscopic:

>15–20 RBCs/hpf Few squamous epithelial cells 0–3 WBCs/hpf Many cystine crystals

- a. What condition does the patient's symptoms represent?
- b. What is the physiologic abnormality causing this condition?
- c. If amino acid chromatography was performed on this specimen, what additional amino acids could be present?
- d. Why are they not present in the microscopic constituents?
- e. What chemical test could be performed to confirm the identity of the cystine crystals?
- f. Based on the family history, what genetic disorder should be considered?

4. An 8-month-old boy is admitted to the pediatric unit with a general diagnosis of failure to thrive. The parents have observed slowness in the infant's development of motor skills. They also mention the occasional appearance of a substance resembling orange sand in the child's diapers. Urinalysis results are as follows:

COLOR: Yellow KETONES: Negative APPEARANCE: Slightly BLOOD: Negative

hazy

SP. GRAVITY: 1.024

BILIRUBIN: Negative

UROBILINOGEN: Normal

PROTEIN: Negative

GLUCOSE: Negative

LEUKOCYTE: Negative

Microscopic:

Many uric acid crystals

- a. Is the urine pH consistent with the appearance of uric acid crystals?
- b. Is there any correlation between the urinalysis results and the substance observed in the child's diapers? Explain your answer.
- c. What disorder do the patient's history and the urinallysis results indicate?
- d. Is the fact that this is a male patient of any significance? Explain your answer.
- e. Name the enzyme that is missing.
- 5. Shortly after arriving for the day shift in the urinalysis laboratory, a technician notices that an undiscarded urine has a black color. The previously completed report indicates the color to be yellow.
 - a. Is this observation significant? Explain your
 - b. What two reactions might be seen with the ferric chloride test?

- c. Which ferric chloride reaction would correlate with a positive Clinitest result?
- d. The original urinalysis report showed the specimen to be positive for ketones. Is this significant? Why or why not?
- 6. Bobby Williams, age 8, is admitted through the emergency department with a ruptured appendix. Although surgery is successful, Bobby's recovery is slow, and the physicians are concerned about his health prior to the ruptured appendix. Bobby's mother states that he has always been noticeably underweight despite a balanced diet and strong appetite and that his younger brother exhibits similar characteristics. A note in his chart from the first postoperative day reports that the evening nurse noticed a purple coloration on the urinary catheter bag.
 - a. Is the catheter bag color significant?
 - b. What additional tests should be run?
 - c. What condition can be suspected from this history?
 - d. What is Bobby's prognosis?
- **7.** A Watson-Schwartz test is performed on an anemic patient who is exhibiting signs of severe photosensitivity. The test result is negative.
 - a. What metabolic disorder was suspected in this patient?
 - b. Was sufficient testing performed to rule out this disorder? Why or why not?
 - c. Can the Watson-Schwartz test be used to detect lead poisoning? Explain your answer.
- **8**. The laboratory receives a request for a resorcinol test.
 - a. What substance will be detected?
 - b. What treatment might this patient be receiving?













Cerebrospinal Fluid

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

- 1 State the three major functions of cerebrospinal fluid (CSF).
- 2 Distribute CSF specimen tubes numbered 1, 2, and 3 to their appropriate laboratory sections and correctly preserve them.
- **3** Describe the appearance of normal CSF.
- 4 Define xanthochromia and state its significance.
- **5** Differentiate between CSF specimens caused by intracranial hemorrhage and a traumatic tap.
- 6 Calculate CSF total, white blood cell (WBC), and red blood cell (RBC) counts when given the number of cells seen, amount of specimen dilution, and the squares counted in the Neubauer chamber.
- **7** Briefly explain the methods used to correct for WBCs and protein that are artificially introduced during a traumatic tap.
- **8** Describe the leukocyte content of the CSF in bacterial, viral, tubercular, and fungal meningitis.
- **9** Describe and give the significance of macrophages in the CSF.
- 10 Differentiate between the appearance of normal choroidal cells and malignant cells.
- 11 State the normal value for CSF total protein.
- 12 List three pathologic conditions that produce an elevated CSF protein.

- Determine whether increased CSF immunoglobulin is the result of damage to the blood-brain barrier or central nervous system production.
- 14 Discuss the significance of CSF electrophoresis findings in multiple sclerosis and the identification of CSF.
- 15 State the normal CSF glucose value.
- **16** Name the possible pathologic significance of a decreased CSF glucose.
- 17 Briefly discuss the diagnostic value of CSF lactate and glutamine determinations.
- **18** Name the microorganism associated with a positive India ink preparation.
- **19** Briefly discuss the diagnostic value of the bacterial and cryptococcal antigen tests.
- 20 Determine whether a suspected case of meningitis is most probably of bacterial, viral, fungal, or tubercular origin, when presented with pertinent laboratory data.
- 21 Describe the role of the Venereal Disease Research Laboratories test and fluorescent treponemal antibody-absorption test for syphilis in CSF testing.
- **22** Describe quality control procedures and safety precautions related to CSF procedures.

KEY TERMS

arachnoid granulations blood-brain barrier choroid plexuses meningitis oligoclonal bands pleocytosis subarachnoid space traumatic tap xanthochromia

■■● Formation and Physiology

First recognized by Cotugno in 1764, cerebrospinal fluid (CSF) is a major fluid of the body. CSF provides a physiologic system to supply nutrients to the nervous tissue, remove metabolic wastes, and produce a mechanical barrier to cushion the brain and spinal cord against trauma.

The brain and spinal cord are lined by the *meninges*, which consists of three layers: the dura mater, *arachnoid*, and pia mater. The outer layer is the dura mater that lines the skull and vertebral canal. The arachnoid is a filamentous (spiderlike) inner membrane. The pia mater is a thin membrane lining the surfaces of the brain and spinal cord.

CSF is produced in the *choroid plexuses* of the two lumbar ventricles and the third and fourth venticles. In adults, approximately 20 mL of fluid is produced every hour. The fluid flows through the *subarachnoid space* located between the arachnoid and pia mater (Fig. 10-1). To maintain a volume of 90 to 150 mL in adults and 10 to 60 mL in neonates, the circulating fluid is reabsorbed back into the blood capillaries in the *arachnoid granulations*/villae at a rate equal to its production. The cells of the arachnoid granulations act as one-way valves that respond to pressure within the central nervous system (CNS) and prevent reflux of the fluid.

The choriod plexuses are capillary networks that form the CSF from plasma by mechanisms of selective filtration under hydrostatic pressure and active transport secretion. Therefore, the chemical composition of the CSF does not resemble an ultrafiltrate of plasma. Capillary walls throughout the body are lined with endothelial cells that are loosely connected to allow passage of soluble nutrients and wastes

between the plasma and tissues. In the choroid plexuses, the endothelial cells have very tight-fitting junctures that prevent the passage of many molecules. This tight-fitting structure of the endothelial cells in the choroid plexuses is termed the *blood-brain barrier*.

Maintaining the integrity of the blood-brain barrier is essential to protect the brain from chemicals and other substances circulating in the blood that could harm the brain tissue. In contrast, the junctures also prevent the passage of helpful substances including antibodies and medications. Disruption of the blood-brain barrier by diseases such as meningitis and multiple sclerosis allows leukocytes, proteins, and additional chemicals to enter the CSF.

Specimen Collection and Handling

CSF is routinely collected by lumbar puncture between the third, fourth, or fifth lumbar vertebrae. Although this procedure is not complicated, it does require certain precautions, including measurement of the intracranial pressure and careful technique to prevent the introduction of infection or the damaging of neural tissue.

The volume of CSF that can be removed is based on the volume available in the patient (adult vs. neonate) and the opening pressure of the CSF taken when the needle first enters the *subarachnoid space*. Elevated pressure requires fluid to be withdrawn slowly, with careful monitoring of the pressure, and may prevent collection of a large volume.

Specimens are collected in three sterile tubes, which are labeled 1, 2, and 3 in the order in which they are withdrawn.

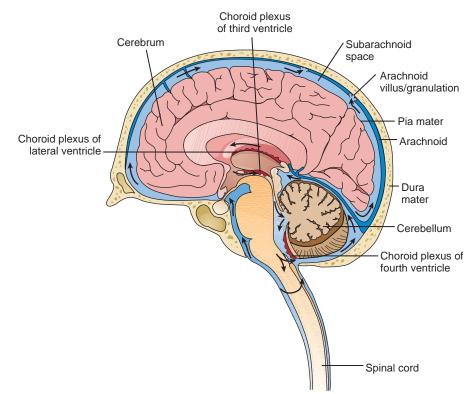


Figure 10–1 The flow of CSF through the brain and spinal column.

Tube 1 is used for chemical and serologic tests because these tests are least affected by blood or bacteria introduced as a result of the tap procedure;

Tube 2 is usually designated for the microbiology laboratory;

Tube 3 is used for the cell count, because it is the least likely to contain cells introduced by the spinal tap procedure.

A fourth tube may be drawn for the microbiology laboratory to provide better exclusion of skin contamination or for additional serologic tests. Supernatant fluid that is left over after each section has performed its tests may also be used for additional chemical or serologic tests. Excess fluid should not be discarded and should be frozen until there is no further use for it (Fig. 10-2).

Considering the discomfort to the patient and the possible complications that can occur during specimen collection, laboratory personnel should handle CSF specimens carefully. Ideally, tests are performed on a STAT basis. If this is not possible, specimens are maintained in the following manner:

- Hematology tubes are refrigerated.
- Microbiology tubes remain at room temperature.
- Chemistry and serology tubes are frozen.

Appearance

The initial appearance of the normally crystal clear CSF can provide valuable diagnostic information. Examination of the fluid occurs first at the bedside and is also included in the laboratory report. The major terminology used to describe CSF

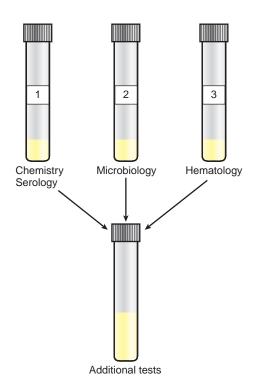


Figure 10–2 CSF specimen collection tubes.

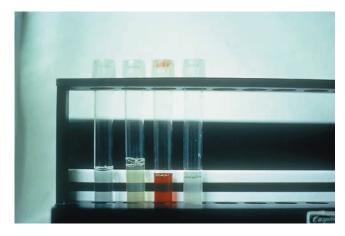


Figure 10–3 Tubes of CSF. Appearance left to right is normal, xanthochromic, hemolyzed, and cloudy.

appearance includes crystal clear, cloudy or turbid, milky, xanthochromic, and hemolyzed/bloody (Fig. 10-3). A cloudy, turbid, or milky specimen can be the result of an increased protein or lipid concentration, but it may also be indicative of infection, with the cloudiness being caused by the presence of WBCs. All specimens should be treated with extreme care because they can be highly contagious; gloves must always be worn and face shields or splash guards should be used while preparing specimens for testing. Fluid for centrifugation must be in capped tubes.

Xanthochromia is a term used to describe CSF supernatant that is pink, orange, or yellow. A variety of factors can cause the appearance of xanthochromia, with the most common being the presence of RBC degradation products. Depending on the amount of blood and the length of time it has been present, the color will vary from pink (very slight amount of oxyhemoglobin) to orange (heavy hemolysis) to yellow (conversion of oxyhemoglobin to unconjugated bilirubin). Other causes of xanthochromia include elevated serum bilirubin, presence of the pigment carotene, markedly increased protein concentrations, and melanoma pigment. Xanthochromia that is caused by bilirubin due to immature liver function is also commonly seen in infants, particularly in those who are premature. The clinical significance of CSF appearance is summarized in Table 10–1.

■■● Traumatic Collection (Tap)

Grossly bloody CSF can be an indication of intracranial hemorrhage, but it may also be due to the puncture of a blood vessel during the spinal tap procedure. Three visual examinations of the collected specimens can usually determine whether the blood is the result of hemorrhage or a *traumatic tap*.

Uneven Distribution of Blood

Blood from a cerebral hemorrhage will be evenly distributed throughout the three CSF specimen tubes, whereas a traumatic tap will have the heaviest concentration of blood in

Table 10-1 Clinical Significance of Cerebrospinal Fluid Appearance			
Appearance	Cause	Major Significance	
Crystal clear		Normal	
Hazy, turbid, milky, cloudy	WBCs	Meningitis	
	Microorganisms	Meningitis	
	Protein	Disorders that affect blood-brain barrier	
		Production of IgG within the CNS	
Oily	Radiographic contrast media		
Bloody	RBCs	Hemorrhage	
		Traumatic tap	
Xanthochromic	Hemoglobin	Old hemorrhage	
		Lysed cells from traumatic tap	
	Bilirubin	RBC degradation	
		Elevated serum bilirubin level	
	Carotene	Increased serum levels	
	Protein	Disorders affecting blood-brain barrier	
	Melanin	Meningeal melanosarcoma	
Clotted	Protein	Disorders affecting blood-brain barrier	
	Clotting factors	Introduced by traumatic tap	
Pellicle	Protein	Disorders that affect blood-brain barrier	
	Clotting factors	Tubercular meningitis	

tube 1, with gradually diminishing amounts in tubes 2 and 3. Performing RBC counts on all three tubes to measure decreasing or constant blood is not always reliable. Streaks of blood also may be seen in specimens acquired following a traumatic procedure.

Clot Formation

Fluid collected from a traumatic tap may form clots owing to the introduction of plasma fibrinogen into the specimen. Bloody CSF caused by intracranial hemorrhage does not contain enough fibrinogen to clot. Diseases in which damage to the blood-brain barrier allows increased filtration of protein and coagulation factors also cause clot formation but do not usually produce a bloody fluid. These conditions include *meningitis*, *Froin syndrome*, and blockage of CSF circulation through the subarachnoid space. A classic web-like pellicle is associated with tubercular meningitis and can be seen after overnight refrigeration of the fluid.³

Xanthochromic Supernatant

RBCs must usually remain in the CSF for approximately 2 hours before noticeable hemolysis begins; therefore, a xanthochromic supernatant would be the result of blood that has been present longer than that introduced by the traumatic tap. Care should be taken, however, to consider this exami-

nation in conjunction with those previously discussed, because a very recent hemorrhage would produce a clear supernatant, and introduction of serum protein from a traumatic tap could also cause the fluid to appear xanthochromic. To examine a bloody fluid for the presence of xanthochromia, the fluid should be centrifuged in a microhematocrit tube and the supernatant examined against a white background.

Additional testing for differentiation includes microscopic examination and the *D-dimer* test. The microscopic finding of macrophages containing ingested RBCs (*ery-throphagocytosis*) or hemosiderin granules is indicative of intracranial hemorrhage. Detection of the fibrin degradation product, D-dimer, by latex agglutination immunoassay indicates the formation of fibrin at a hemorrhage site.

■■● Cell Count

The cell count that is routinely performed on CSF specimens is the leukocyte (WBC) count. As discussed previously, the presence and significance of RBCs can usually be ascertained from the appearance of the specimen. Therefore, RBC counts are usually determined only when a traumatic tap has occurred and a correction for leukocytes or protein is desired. The RBC count can be calculated by performing a total cell count and a WBC count and subtracting the WBC count from the total count, if necessary. Any cell count should be performed immediately, because WBCs (particularly granulo-

cytes) and RBCs begin to lyse within 1 hour, with 40% of the leukocytes disintegrating after 2 hours.⁴ Specimens that cannot be analyzed immediately should be refrigerated.

Methodology

Normal adult CSF contains 0 to 5 WBCs/µL. The number is higher in children, and as many as 30 mononuclear cells/µL can be considered normal in newborns. Specimens that contain up to 200 WBCs or 400 RBCs/µl may appear clear, so it is necessary to examine all specimens microscopically. An improved Neubauer counting chamber (Fig. 10-4) is routinely used for performing CSF cell counts. Traditionally, electronic cell counters have not been used for performing CSF cell counts, owing to high background counts and poor reproducibility of low counts.

The Advia 120 Hematology System (Siemens Medical Solutions Diagnostics, Tarrytown, N.Y.) has received Food and Drug Administration (FDA) approval for addition of a CSF assay. The instrument performs WBC counts on all samples, RBC counts on samples with less than 1500 cells/ μ L, and differential counts for neutrophils, lymphocytes, and monocytes.

Calculation of CSF Cell Counts

The standard Neubauer calculation formula used for blood cell counts is also applied to CSF cell counts to determine the number of cells per microliter.

$$\frac{\text{Number of cells counted} \times \text{dilution}}{\text{Number of squares counted} \times \text{volume of 1 square}} = \text{cells/}\mu\text{L}$$

This formula can be used for both diluted and undiluted specimens and offers flexibility in the number and size of the

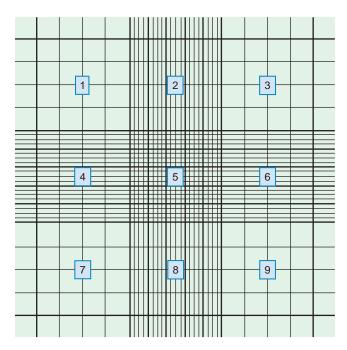


Figure 10–4 Neubauer counting chamber depicting the nine large square counting areas.

squares counted. Many varied calculations are available, including condensations of the formula to provide single factors by which to multiply the cell count. Keep in mind that the purpose of any calculation is to convert the number of cells counted in a specific amount of fluid to the number of cells that would be present in 1 μ L of fluid. Therefore, a factor can be used only when the dilution and counting area are specific for that factor.

The methodology presented in this chapter eliminates the need to correct for the volume counted by counting the four large corner squares (0.4 μ L) and the large center square (0.1 μ L) on each side of the counting chamber.⁷

Example Number of cells counted × dilution × $\frac{1 \mu L}{1 \mu L (0.1 \times 10)}$ = cells/ μL (volume counted)

Total Cell Count

Clear specimens may be counted undiluted, provided no overlapping of cells is seen during the microscopic examination. When dilutions are required, calibrated automatic pipettes, not mouth pipetting, are used. Dilutions for total cell counts are made with normal saline, mixed by inversion, and loaded into the hemocytometer with a Pasteur pipette. Cells are counted in the four corner squares and the center square on both sides of the hemocytometer. As shown in the preceding example, the number of cells counted multiplied by the dilution factor equals the number of cells per microliter.

WBC Count

Lysis of RBCs must be obtained prior to performing the WBC count on either diluted or undiluted specimens. Specimens requiring dilution can be diluted in the manner described previously, substituting 3% glacial acetic acid to lyse the RBCs. Addition of methylene blue to the diluting fluid stains the WBCs, providing better differentiation between neutrophils and mononuclear cells.

To prepare a clear specimen that does not require dilution for counting, place four drops of mixed specimen in a clean tube. Rinse a Pasteur pipette with 3% glacial acetic acid, draining thoroughly, and draw the four drops of CSF into the rinsed pipette. Allow the pipette to sit for 1 minute, mix the solution in the pipette, discard the first drop, and load the hemocytometer. As in the total cell count, WBCs are counted in the four corner squares, and the center square on both sides of the hemocytometer and the number is multiplied by the dilution factor to obtain the number of WBCs per microliter. If a different number of squares is counted, the standard Neubauer formula should be used to obtain the number of cells per microliter.

Corrections for Contamination

Calculations are possible to correct for WBCs and protein artificially introduced into the CSF as the result of a traumatic tap. Determination of the CSF RBC count and the blood RBC and WBC counts is necessary to perform the correction. By determining the ratio of WBCs to RBCs in the peripheral blood and comparing this ratio with the number of contaminating RBCs, the number of artificially added WBCs can be calculated using the following formula:

WBC (added) =
$$\frac{\text{WBC (blood)} \times \text{RBC (CSF)}}{\text{RBC (blood)}}$$

An approximate CSF WBC count can then be obtained by subtracting the "added" WBCs from the actual count. When peripheral blood RBC and WBC counts are in the normal range, many laboratories choose to simply subtract 1 WBC for every 700 RBCs present in the CSF. Studies have shown a high percentage of error in the correction of fluids containing a large number of RBCs, indicating correction may be of little value under these circumstances.⁸

Quality Control of Cerebrospinal Fluid and Other Body Fluid Cell Counts

Liquid commercial controls for spinal fluid RBC and WBC counts are available from several manufacturers. They can be purchased at two levels of concentration. In-house controls can also be prepared.

On a biweekly basis, all diluents should be checked for contamination by examination in a counting chamber under $4 \times \text{magnification}$. Contaminated diluents should be discarded and new solutions prepared.

On a monthly basis, the speed of the cytocentrifuge should be checked with a tachometer, and the timing should be checked with a stopwatch.

If nondisposable counting chambers are used, they must be soaked in a bactericidal solution for at least 15 minutes and then thoroughly rinsed with water and cleaned with isopropyl alcohol.

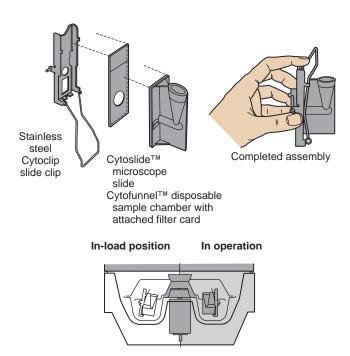
Differential Count on a Cerebrospinal Fluid Specimen

Identifying the type or types of cells present in the CSF is a valuable diagnostic aid. The differential count should be performed on a stained smear and not from the cells in the counting chamber. Poor visualization of the cells as they appear in the counting chamber has led to the laboratory practice of reporting only the percentage of mononuculear and polynuclear cells present, and this can result in the overlooking of abnormal cells with considerable diagnostic importance. To ensure that the maximum number of cells are available for examination, the specimen should be concentrated prior to the preparation of the smear.

Methods available for specimen concentration include sedimentation, filtration, centrifugation, and cytocentrifugation. Sedimentation and filtration are not routinely used in the clinical laboratory, although they do produce less cellular distortion. Most laboratories that do not have a cytocentrifuge concentrate specimens with routine centrifugation. The specimen is centrifuged for 5 to 10 minutes, supernatant fluid is removed and saved for additional tests, and slides made from the suspended sediment are allowed to air dry and are stained with Wright's stain. When the differential count is performed, 100 cells should be counted, classified, and reported in terms of percentage. If the cell count is low and finding 100 cells is not possible, report only the numbers of the cell types seen.

Cytocentrifugation

A diagramatic view of the principle of cytocentrifugation is shown in Figure 10-5. Fluid is added to the conical chamber, and as the specimen is centrifuged, cells present in the fluid are forced into a monolayer within a 6-mm diameter circle on the slide. Fluid is absorbed by the filter paper blotter, producing a more concentrated area of cells. As little as 0.1 mL of CSF combined with one drop of 30% albumin produces an adequate cell yield when processed with the cytocentrifuge. Addition of albumin increases the cell yield and decreases the cellular distortion frequently seen on cytocentrifuged specimens. Positively charged coated slides to attract cells (Shandon, Inc, Pittsburgh, Pa.) are also available. Cellular distortion may include cytoplasmic vacuoles, nuclear clefting, prominent nucleoli, indistinct nuclear and cytoplasmic borders and cellular clumping that resembles malignancy. Cells from both the center and periphery of the slide should be examined because cellular characteristics may vary between areas of the slide.



This cutaway drawing illustrates, at left, the in-load position which shows the sample chamber assembly in a tilted-back position, so that the sample is not absorbed by the filter card. During spinning, centrifugal force tilts the assembly upright and forces the sample to flow toward the microscope slide.

Figure 10–5 Cytospin 3 cytocentrifuge specimen processing assembly (Courtesy of Shandon, Inc., Pittsburgh, Pa.).

Table 10-2 Cytocentrifuge Recovery Chart ⁷		
Number of WBCs Counted in Chamber	Number of Cells Counted on Cytocentrifuge Slide	
0	0–40	
1–5	20–100	
6–10	60–150	
11–20	150–250	
20	250	

A daily control slide for bacteria should also be prepared using 0.2 mL saline and two drops of the 30% albumin currently being used. The slide is stained and examined if bacteria are seen on a patient's slide.

In Table 10–2, a cytocentrifuge recovery chart is provided for comparison with chamber counts. The chamber count should be repeated if too many cells are seen on the slide, and a new slide should be prepared if not enough cells are seen on the slide.⁷

Cerebrospinal Fluid Cellular Constituents

The cells found in normal CSF are primarily lymphocytes and monocytes (Figs. 10-6 and 10-7). Adults usually have a predominance of lymphocytes to monocytes (70:30), whereas the ratio is essentially reversed in children. 5 Improved concentration methods are also showing occasional neutrophils in normal CSF.9 The presence of increased numbers of these normal cells (termed *pleocytosis*) is considered abnormal, as is the finding of immature leukocytes, eosinophils, plasma cells, macrophages, increased tissue cells, and malignant cells.

When pleocytosis involving neutrophils, lymphocytes, or monocytes is present, the CSF differential count is most frequently associated with its role in providing diagnostic information about the type of microorganism that is causing an infection of the meninges (meningitis). A high CSF WBC count of which the majority of the cells are neutrophils is considered indicative of bacterial meningitis. Likewise, a moderately elevated CSF WBC count with a high percentage of lymphocytes and monocytes suggests meningitis of viral, tubercular, fungal, or parasitic origin.

As seen in Table 10–3, many pathologic conditions other than meningitis can be associated with the finding of abnor-

Table 10-3 Predominant Cells Seen in Cerebrospinal Fluid			
Type of Cell	Major Clinical Significance	Microscopic Findings	
Lymphocytes	Normal Viral, tubercular, and fungal meningitis Multiple sclerosis	All stages of development may be found	
Neutrophils	Bacterial meningitis Early cases of viral, tubercular, and fungal meningitis Cerebral hemorrhage	Granules may be less prominent than in blood Cells disintegrate rapidly	
Monocytes	Normal Viral, tubercular, and fungal meningitis Multiple sclerosis	Found mixed with lymphocytes	
Macrophages	RBCs in spinal fluid Contrast media	May contain phagocytized RBCs appearing as empty vacuoles or ghost cells, hemosiderin granules and hematoidin crystals	
Blast forms	Acute leukemia	Lymphoblasts, myeloblasts, or monoblasts	
Lymphoma cells	Disseminated lymphomas	Resemble lymphocytes with cleft nuclei	
Plasma cells	Multiple sclerosis Lymphocyte reactions	Traditional and classic forms seen	
Ependymal, choroidal, and spindle-shaped cells	Diagnostic procedures	Seen in clusters with distinct nuclei and distinct cell walls	
Malignant cells	Metastatic carcinomas Primary central nervous system carcinoma	Seen in clusters with fusing of cell borders and nuclei	

184 CHAPTER IO • Cerebrospinal Fluid

mal cells in the CSF. Therefore, because laboratory personnel become so accustomed to finding neutrophils, lymphocytes, and monocytes, they should be careful not to overlook other types of cells. Cell forms differing from those found in blood include macrophages, choroid plexus and ependymal cells, spindle-shaped cells, and malignant cells.

Neutrophils

In additon to bacterial meningitis, increased neutrophils also are seen in the early stages (1 to 2 days) of viral, fungal, tubercular, and parasitic meningitis. Neutrophils may also contain cytoplasmic vacuoles following cytocentrifugation (Fig. 10-8). Granules are also lost more rapidly in CSF. Neutrophils associated with bacterial meningitis may contain phagocytized bacteria (Figs. 10-9 and 10-10). Although of little clinical significance, neutrophils may be increased following central nervous system (CNS) hemorrhage, repeated lumbar punctures, and injection of medications or radiographic dye.

Neutrophils with *pyknotic* nucleii indicate degenerating cells. They may resemble nucleated red blood cells (NRBCs) but usually have multiple nucleii. When a single nucleus is present they can appear similar to NRBCs (Fig. 10-11). NRBCs are seen as a result of bone marrow contamination during the spinal tap (Figs. 10-12 and 10-13). This is found in approximately 1% of specimens. ¹⁰ Capillary structures and endothelial cells may be seen following a traumatic tap (Fig. 10-14).

Lymphocytes and Monocytes

A mixture of lymphocytes and monocytes is common in cases of viral, tubercular, and fungal meningitis. Reactive lymphocytes containing increased dark blue cytoplasm and clumped chromatin are frequently present during viral infections in conjunction with normal cells (Fig. 10-15). Increased lymphocytes are seen in cases of both asymptomatic HIV infection and AIDS. A moderately elevated WBC count (less than 50 WBCs/ μ L) with increased normal and reactive lymphocytes and plasma cells may be indicative of multiple sclerosis or other degenerative neurologic disorders. 11

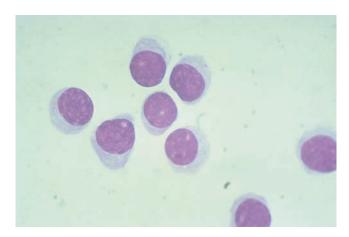


Figure 10–6 Normal lymphocytes. Some cytocentrifuge distortion of cytoplasm (×1000).

Eosinophils

Increased eosinophils are seen in the CSF in association with parasitic infections, fungal infections (primarily *Coccidioides immitis*), and introduction of foreign material, including medications and shunts into the CNS (Fig. 10-16).

Macrophages

The purpose of macrophages in the CSF is to remove cellular debris and foreign objects such as RBCs. Macrophages appear within 2 to 4 hours after RBCs enter the CSF and are frequently seen following repeated taps. They tend to have more cytoplasm than monocytes in the peripheral blood (PB) (Fig. 10-17).

The finding of increased macrophages is indicative of a previous hemorrhage (Fig. 10-18). Further degradation of the phagocytized RBCs results in the appearance of dark blue or black iron-containing hemosiderin granules (Figs. 10-19 through 10-22). Yellow *hematoidin* crystals represent further degeneration. They are iron-free, consisting of hemoglobin and unconjugated bilirubin (Figs. 10-23 and 10-24).

Nonpathologically Significant Cells

These cells are most frequently seen following diagnostic procedures such as pneumoencephalography and in fluid obtained from ventricular taps or during neurosurgery. The cells often appear in clusters and can be distinguished from malignant cells by their uniform appearance.

Choroidal cells are from the epithelial lining of the choroid plexus. They are seen singularly and in clumps. Nucleoli are usually absent and nuclei have a uniform appearance (Fig. 10-25).

Ependymal cells are from the lining of the ventricles and neural canal. They have less defined cell membranes and are frequently seen in clusters. Nucleoli are often present (Fig. 10-26).

Spindle-shaped cells represent lining cells from the arachnoid. They are ususally seen in clusters and may be seen with systemic malignancies (Fig. 10-27).

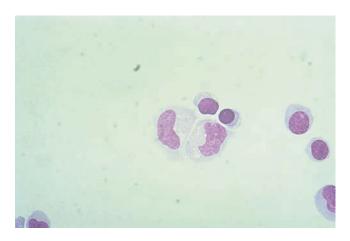


Figure 10–7 Normal lymphocytes and monocytes (×500).

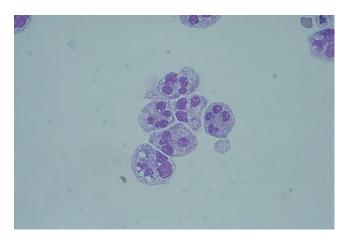


Figure 10–8 Neutrophils with cytoplasmic vacuoles resulting from cytocentrifugation (×500).

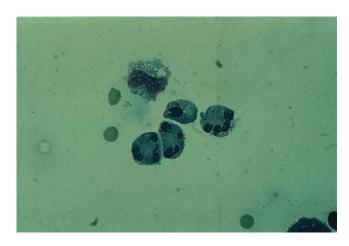


Figure 10–11 Neutrophils with pyknotic nuclei. Notice the cell with a single nucleus in the center (×1000).

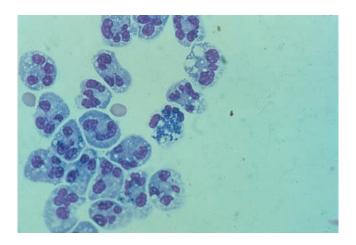


Figure 10–9 Neutrophils with intracellular bacteria (×1000).

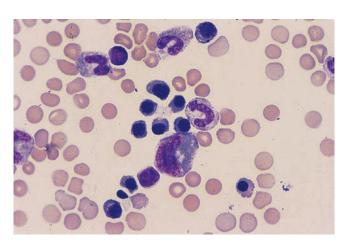


Figure 10–12 Nucleated RBCs seen with bone marrow contamination (\times 1000).

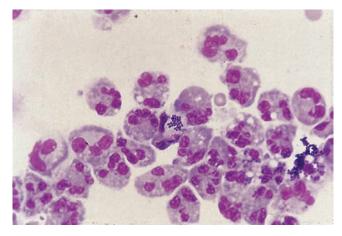


Figure 10–10 Neutrophils with intracellular and extracellular bacteria (×1000).

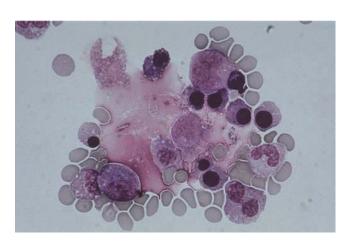


Figure 10–13 Bone marrow contamination (\times 1000). Notice the immature RBCs and granulocytes.

186 CHAPTER 10 • Cerebrospinal Fluid

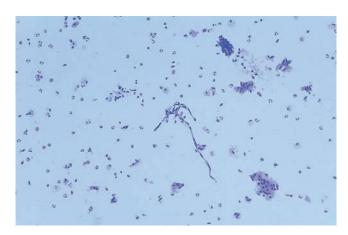


Figure 10–14 Capillary and tissue fragments from a traumatic tap $(\times 100)$.

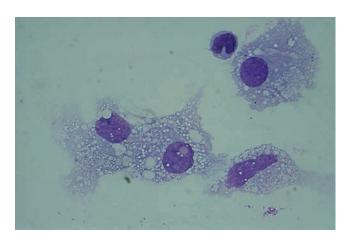


Figure 10–17 Macrophages. Notice the large amount of cytoplasm and vacuoles (×500).

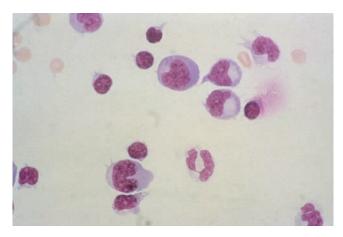


Figure 10–15 Broad spectrum of lymphocytes and monocytes in viral meningitis (×1000).

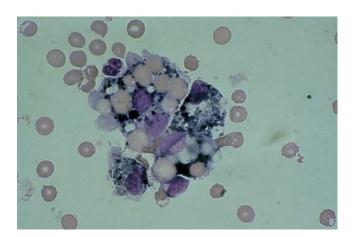


Figure 10–18 Macrophages showing erythrophagocytosis (×500).

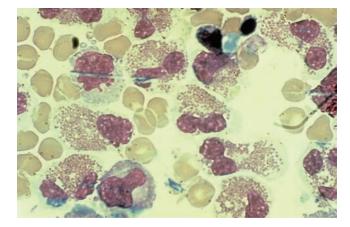


Figure 10–16 Eosinophils (\times 1000). Notice cytocentrifuge distortion.

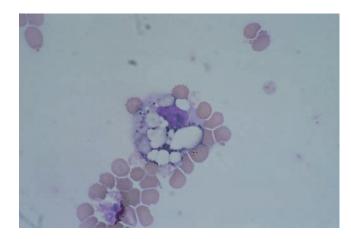


Figure 10–19 Macrophage with RBC remnants (×500).

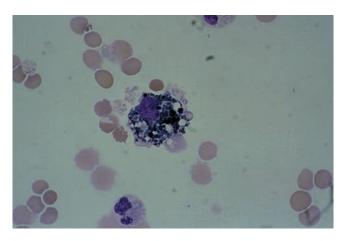


Figure 10–20 Macrophage with aggregated hemosiderin granules (×500).

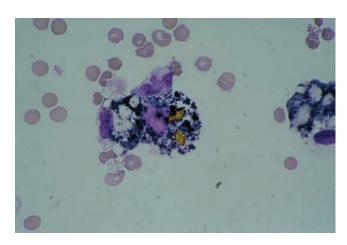


Figure 10–23 Macrophage containing hemosiderin and hematoidin crystals (×500).

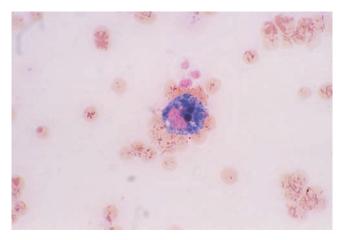


Figure 10–21 Macrophage containing hemosiderin stained with Prussian blue (×250).

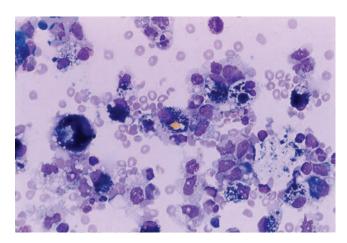


Figure 10–24 Macrophages with hemosiderin and hematoidin (×250). Notice the bright yellow color.



Figure 10–22 Macrophage with coarse hemosiderin granules $(\times 500)$.

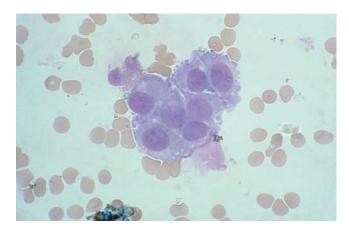


Figure 10–25 Choroidal cells showing distinct cell borders and nuclear uniformity $(\times 500)$.

188 CHAPTER IO • Cerebrospinal Fluid

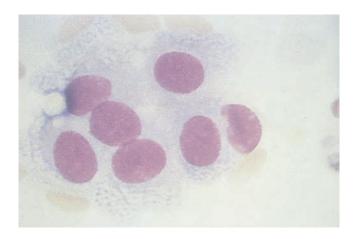


Figure 10–26 Ependymal cells. Notice the nucleoli and less distinct cell borders (×1000).

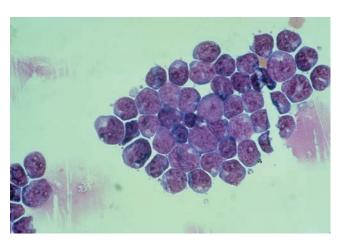


Figure 10–28 Lymphoblasts from acute lymphocytic leukemia (×500).

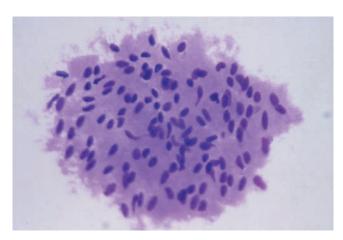


Figure 10–27 Cluster of spindle-shaped cells (×500).

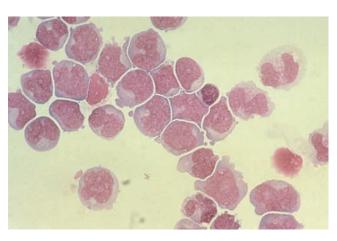


Figure 10–29 Myeloblasts from acute myelocytic leukemia (×500).

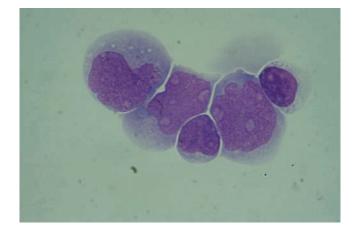


Figure 10–30 Monoblasts and two lymphocytes (×1000). Notice the prominent nucleoli.

Malignant Cells of Hematologic Origin

Lymphoblasts, myeloblasts, and monoblasts (Figs 10-28 to 10-30) in the CSF are frequently seen as a serious complication of acute leukemias. Nucleoli are often more prominent than in blood smears.

Lymphoma cells are also seen in the CSF indicating dissemination from the lymphoid tissue. They resemble large and small lymphocytes and usually appear in clusters of large, small, or mixed cells based on the classification of the lymphoma. Nuclei may appear cleaved, and prominent nucleoli are present (Figs. 10-31 to 10-33).

Malignant Cells of Nonhematologic Origin

Metastatic carcinoma cells of nonhematologic origin are primarily from lung, breast, renal, and gastrointestinal malig-

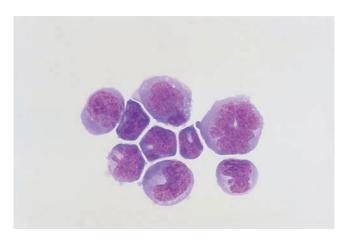


Figure 10–31 Cleaved and noncleaved lymphoma cells $(\times 1000)$.

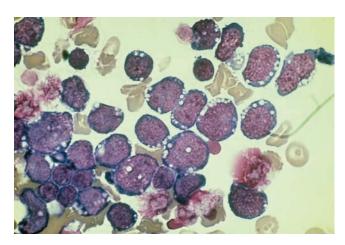


Figure 10–33 Burkitt lymphoma. Notice characteristic vacuoles (×500).

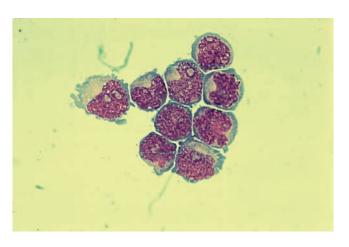


Figure 10–32 Lymphoma cells with nucleoli (×500).

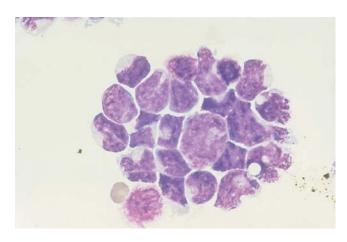


Figure 10–34 Medulloblastoma (×1000). Notice cellular clustering, nuclear irregularities, and rosette formation.

nancies. Cells from primary CNS tumors include *astrocytomas*, *retinoblastomas*, and *medulloblastomas* (Fig. 10-34). They usually appear in clusters and must be distinguished from normal clusters of ependymal, choroid plexus, lymphoma, and leukemia cells. Fusing of cell walls and nuclear irregularities and hyperchromatic nucleoli are seen in clusters of malignant cells. Slides containing abnormal cells must be referred to pathology.

Chemistry Tests

Because CSF is formed by filtration of the plasma, one would expect to find the same low-molecular-weight chemicals in the CSF that are found in the plasma. This is essentially true; however, because the filtration process is selective and the chemical composition is controlled by the blood-brain barrier, normal values for CSF chemicals are not the same as the

plasma values. Abnormal values result from alterations in the permeability of the blood-brain barrier or increased production or metabolism by the neural cells in response to a pathologic condition. They seldom have the same diagnostic significance as plasma abnormalities. The clinically important CSF chemicals are few; under certain conditions it may be necessary to measure a larger variety. Many CSF metabolites are currently under investigation to determine their possible diagnostic significance.

Cerebrospinal Protein

The most frequently performed chemical test on CSF is the protein determination. Normal CSF contains a very small amount of protein. Normal values for total CSF protein are usually listed as 15 to 45 mg/dL, but are somewhat method dependent, and higher values are found in infants

190 CHAPTER IO • Cerebrospinal Fluid

and older persons. 12 This value is reported in milligrams per deciliter and not grams per deciliter, as are plasma protein concentrations.

In general, the CSF contains protein fractions similar to those found in serum; however, as can be seen in Table 10–4, the ratio of CSF proteins to serum proteins varies among the fractions. As in serum, albumin makes up the majority of CSF protein. But in contrast to serum, prealbumin is the second most prevalent fraction in CSF. The alpha globulins include primarily haptoglobin and ceruloplasmin. Transferrin is the major beta globulin present; also, a separate carbohydrate-deficient transferrin fraction, referred to as "tau," is seen in CSF and not in serum. CSF gamma globulin is primarily immunoglobulin G (IgG), with only a small amount of IgA. Immunoglobulin M (IgM), fibrinogen, and beta lipoprotein are not found in normal CSE.¹³

Clinical Significance of Elevated Protein Values

Elevated total protein values are most frequently seen in pathologic conditions. Abnormally low values are present when fluid is leaking from the CNS. The causes of elevated CSF protein include damage to the blood-brain barrier, production of immunoglobulins within the CNS, decreased clearance of normal protein from the fluid, and degeneration of neural tissue. Meningitis and hemorrhage conditions that damage the blood-brain barrier are the most common causes of elevated CSF protein. Many other neurologic disorders can elevate the CSF protein, and finding an abnormal result on clear fluid with a low cell count is not unusual (Table 10–5).

Artificially Induced Plasma Proteins

Table 10-4

Immunoglobulin A

Plasma protein can be artificially introduced into a specimen by a traumatic tap in the same manner as blood cells. A correction calculation similar to that used in cell counts is available for protein measurements; however, if the correction is to

Cerebrospinal Fluid

and Serum Protein Correlations			
	Fluid (mg/dL)	Cerebrospinal (mg/dL)	Serum Ratio
Prealbumin	1.7	23.8	14
Albumin	15.5	3600	236
Ceruloplasmin	0.1	36.6	366
Transferrin	1.4	204	142
Immunoglobulin G	1.2	987	802

Adapted from Fishman, RA: Cerebrospinal fluid in diseases of the nervous system, 2nd ed, WB Saunders, Philadelphia, 1992.

0.13

175

1346

Table 10–5

Clinical Causes of Abnormal CSF Protein Values

Elevated Results

- Meningitis
- Hemorrhage
- Primary CNS tumors
- Multiple sclerosis
- Guillain-Barré syndrome
- Neurosyphilis
- Polyneuritis
- Myxedema
- Cushing disease
- Connective tissue disease
- Polyneuritis
- Diabetes
- Uremia

Decreased Results

- CSF leakage/trauma
- Recent puncture
- Rapid CSF production
- Water intoxication

be used, both the cell count and the protein determination must be done on the same tube.⁶ When the blood hematocrit and serum protein values are normal, subtracting 1 mg/dL of protein for every 1200 RBCs counted is acceptable.

[serum protein mg/dL
$$\times$$
 (1.00 - Hct)] \times CSF RBCs/ μ L mg/dL protein added = $\frac{(plasma\ volume)}{blood\ RBCs/\mu L}$

Methodology

The two most routinely used techniques for measuring total CSF protein use the principles of turbidity production or dyebinding ability. The turbidity method has been adapted to automated instrumentation in the form of nephelometry. Methods for the measurement of CSF protein are available for most automated chemistry analyzers.

Protein Fractions

Routine CSF protein procedures are designed to measure total protein concentration. However, diagnosis of neurologic disorders associated with abnormal CSF protein often requires measurement of the individual protein fractions. Protein that appears in the CSF as a result of damage to the integrity of the blood-brain barrier contains fractions proportional to those in plasma, with albumin present in the highest concentration. Diseases, including multiple sclerosis, that stimulate the immunocompetent cells in the CNS show a higher proportion of IgG.

To accurately determine whether IgG is increased because it is being produced within the CNS or is elevated as the result of a defect in the blood-brain barrier, comparisons between serum and CSF levels of albumin and IgG must be made. Methods include the CSF/serum albumin index to evaluate the integrity of the blood-brain barrier and the CSF IgG index to measure IgG synthesis within the CNS.

The CSF/serum albumin index is calculated after determining the concentration of CSF albumin in milligrams per deciliter and the serum concentration in grams per deciliter. The formula used is as follows:

$$CSF/serum albumin index = \frac{CSF albumin (mg/dL)}{Serum albumin (g/dL)}$$

An index value less than 9 represents an intact bloodbrain barrier. The index increases relative to the amount of damage to the barrier.

Calculation of an IgG index, which is actually a comparison of the CSF/serum albumin index with the CSF/ serum IgG index, compensates for any IgG entering the CSF via the blood-brain barrier. ¹⁴ It is performed by dividing the CSF/serum IgG index by the CSF/serum albumin index as follows:

$$IgG \ index = \frac{CSF \ IgG \ (mg/dL)/serum \ IgG \ (g/dL)}{CSF \ albumin \ (mg/dL)/serum \ albumin \ (g/dL)}$$

Normal IgG index values vary slightly among laboratories; however, in general, values greater than 0.70 are indicative of IgG production within the CNS.

Techniques for the measurement of CSF albumin and globulin have been adapted to automated instrumentation.

Electrophoresis

The primary purpose for performing CSF protein electrophoresis is for the detection of *oligoclonal bands* representing inflammation within the CNS. The bands are located in the gamma region of the protein electrophoresis, indicating immunoglobulin production. To ensure that the oligoclonal bands are present as the result of neurologic inflammation, simultaneous serum electrophoresis must be performed. Disorders such as leukemia, lymphoma, and viral infections may produce serum banding, which can appear in the CSF as a result of blood-brain barrier leakage or traumatic introduction of blood into the CSF specimen. Banding representing both systemic and neurologic involvement is seen in the serum and CSF with HIV infection.¹⁵

The presence of two or more oligoclonal bands in the CSF that are not present in the serum can be a valuable tool in the diagnosis of multiple sclerosis, particularly when accompanied by an increased IgG index. Other neurologic disorders including encephalitis, neurosyphilis, *Guillain-Barré* syndrome, and neoplastic disorders also produce oligoclonal banding that may not be present in the serum. Therefore, the presence of oligoclonal banding must be considered in conjunction with clinical symptoms. Oligoclonal banding remains positive during remission of multiple sclerosis, but disappears in other disorders. ¹¹

Low protein levels in the CSF make concentration of the fluid prior to performing electrophoresis essential for most electrophoretic techniques. Agarose gel electrophoresis followed by Coomassie brilliant blue staining is most frequently performed in the clinical laboratory. Better resolution can be obtained using immunofixation electrophoresis (IFE) and isoelectric focusing (IEF) followed by silver staining. Specimen concentration is not required by the more sensitive IEF procedure.

Electrophoresis is also the method of choice when determining if a fluid is actually CSF. Identification can be made based on the appearance of the previously mentioned extra isoform of transferrin, tau, that is found only in CSF.¹⁶

Myelin Basic Protein

The presence of myelin basic protein (MBP) in the CSF is indicative of recent destruction of the myelin sheath that protects the axons of the neurons (*demyelination*). Measurement of the amount of MBP in the CSF can be used to monitor the course of multiple sclerosis.¹⁷ It may also provide a valuable measure of the effectiveness of current and future treatments. Immunoassay techniques are used for measurement.¹⁸

Cerebrospinal Fluid Glucose

Glucose enters the CSF by selective transport across the blood-brain barrier, which results in a normal value that is approximately 60% to 70% that of the plasma glucose. If the plasma glucose is 100 mg/dL, then a normal CSF glucose would be approximately 65 mg/dL. For an accurate evaluation of CSF glucose, a blood glucose test must be run for comparison. The blood glucose should be drawn about 2 hours prior to the spinal tap to allow time for equilibration between the blood and fluid. CSF glucose is analyzed using the same procedures employed for blood glucose. Specimens should be tested immediately because glycolysis occurs rapidly in the CSF.

The diagnostic significance of CSF glucose is confined to the finding of values that are decreased in relation to plasma values. Elevated CSF glucose values are always a result of plasma elevations. Low CSF glucose values can be of considerable diagnostic value in determining the causative agents in meningitis. The finding of a markedly decreased CSF glucose accompanied by an increased WBC count and a large percentage of neutrophils is indicative of bacterial meningitis. If the WBCs are lymphocytes instead of neutrophils, tubercular meningitis is suspected. Likewise, if a normal CSF glucose value is found with an increased number of lymphocytes, the diagnosis would favor viral meningitis. Classic laboratory patterns such as those just described may not be found in all cases of meningitis, but they can be helpful when they are present.

Decreased CSF glucose values are caused primarily by alterations in the mechanisms of glucose transport across the blood-brain barrier and by increased use of glucose by the brain cells. The common tendency to associate the decreased glucose totally with its use by microorganisms and leukocytes

cannot account for the variations in glucose concentrations seen in different types of meningitis and the decreased levels seen in other disorders producing damage to the CNS. ¹⁹

Cerebrospinal Fluid Lactate

The determination of CSF lactate levels can be a valuable aid in the diagnosis and management of meningitis cases. In bacterial, tubercular, and fungal meningitis, the elevation of CSF lactate to levels greater than 25 mg/dL occurs much more consistently than does the depression of glucose and provides more reliable information when the initial diagnosis is difficult. Levels greater than 35 mg/dL are frequently seen with bacterial meningitis, whereas in viral meningitis, lactate levels remain lower than 25 mg/dL. CSF lactate levels remain elevated during initial treatment but fall rapidly when treatment is successful, thus offering a sensitive method for evaluating the effectiveness of antibiotic therapy.

Destruction of tissue within the CNS owing to oxygen deprivation (*hypoxia*) causes the production of increased CSF lactic acid levels. Therefore, elevated CSF lactate is not limited to meningitis and can result from any condition that decreases the flow of oxygen to the tissues. CSF lactate levels are frequently used to monitor severe head injuries. RBCs contain high concentrations of lactate, and falsely elevated results may be obtained on xanthochromic or hemolyzed fluid.⁹

Cerebrospinal Fluid Glutamine

Glutamine is produced from ammonia and α -ketoglutarate by the brain cells. This process serves to remove the toxic metabolic waste product ammonia from the CNS. The normal concentration of glutamine in the CSF is 8 to 18 mg/dL. ²⁰ Elevated levels are found in association with liver disorders that result in increased blood and CSF ammonia. Increased synthesis of glutamine is caused by the excess ammonia that

is present in the CNS; therefore, the determination of CSF glutamine provides an indirect test for the presence of excess ammonia in the CSF. Several methods of assaying glutamine are available and are based on the measurement of ammonia liberated from the glutamine. This is preferred over the direct measurement of CSF ammonia because the concentration of glutamine remains more stable than the concentration of volatile ammonia in the collected specimen. The CSF glutamine level also correlates with clinical symptoms much better than does the blood ammonia.²⁰

As the concentration of ammonia in the CSF increases, the supply of α -ketoglutarate becomes depleted; glutamine can no longer be produced to remove the toxic ammonia, and coma ensues. Some disturbance of consciousness is almost always seen when glutamine levels are more than 35 mg/dL. ¹³ Therefore, the CSF glutamine test is a frequently requested procedure for patients with coma of unknown origin. Approximately 75% of children with *Reye syndrome* have elevated CSF glutamine levels. ²¹

Microbiology Tests

The role of the microbiology laboratory in the analysis of CSF lies in the identification of the causative agent in meningitis. For positive identification, the microorganism must be recovered from the fluid by growing it on the appropriate culture medium. This can take anywhere from 24 hours in cases of bacterial meningitis to 6 weeks for tubercular meningitis. Consequently, in many instances, the CSF culture is actually a confirmatory rather than a diagnostic procedure. However, the microbiology laboratory does have several methods available to provide information for a preliminary diagnosis. These methods include the Gram stain, acid-fast stain, India ink preparation, and latex agglutination tests. In Table 10–6, the laboratory tests used in the differential diagnosis of meningitis are compared.

Table 10-6 Major Laboratory Results for the Differential Diagnosis of Meningitis			
Bacterial	Viral	Tubercular	Fungal
Elevated WBC count	Elevated WBC count	Elevated WBC count	Elevated WBC count
Neutrophils present	Lymphocytes present	Lymphocytes and monocytes present	Lymphocytes and monocytes present
Marked protein elevation	Moderate protein elevation	Moderate to marked protein elevation	Moderate to marked protein elevation
Markedly decreased glucose level	Normal glucose level	Decreased glucose level	Normal to decreased glucose level
Lactate level >35 mg/dL	Normal lactate level	Lactate level>25 mg/dL Pellicle formation	Lactate level >25 mg/dL Positive India ink with Cryptococcus neoformans
Positive Gram stain and bacterial antigen tests			Positive immunologic test for <i>C. neoformans</i>

Summary of Cerebrospinal Fluid Chemistry Tests

Protein

- I. Normal concentration is 15 to 45 mg/dL.
- 2. Elevated values are most frequently seen in patients with meningitis, hemorrhage, and multiple sclerosis.

Glucose

- I. Normal value is 60% to 70% of the plasma concentration
- 2. Decreased levels are seen in patients with bacterial, tubercular, and fungal meningitis.

Lactate

- Levels >35 mg/dL are seen in patients with bacterial meningitis.
- 2. Levels >25 mg/dL are found in patients with tubercular and fungal meningitis.
- 3. Lower levels are seen in patients with viral meningitis.

Glutamine

- 1. Normal concentration is 8 to 18 mg/dL.
- Levels >35 mg/dL are associated with some disturbance of consciousness.

Gram Stain

The Gram stain is routinely performed on CSF from all suspected cases of meningitis, although its value lies in the detection of bacterial and fungal organisms. All smears and cultures should be performed on concentrated specimens because often only a few organisms are present at the onset of the disease. The CSF should be centrifuged at 1500 g for 15 minutes, and slides and cultures should be prepared from the sediment.²³ Use of the cytocentrifuge provides a highly concentrated specimen for Gram stains. Even when concentrated specimens are used, at least a 10% chance exists that Gram stains and cultures will be negative. Thus, blood cultures should be taken, because the causative organism is often present in both the CSF and the blood.9 A CSF Gram stain is one of the most difficult slides to interpret because the number of organisms present is usually small, and they can easily be overlooked, resulting in a false-negative report. Also, false-positive reports can occur if precipitated stain or debris is mistaken for microorganisms. Therefore, considerable care should be taken when interpreting a Gram stain. Organisms most frequently encountered include Streptococcus pneumoniae (gram-positive cocci), Haemophilus influenzae (pleomorphic gram-negative rods), Escherichia coli (gramnegative rods), and Neisseria meningitidis (gram-negative cocci). The gram-positive cocci, Streptococcus agalactiae and the gram-positive rods Listeria monocytogenes may be encountered in newborns.

Acid-fast or fluorescent antibody stains are not routinely performed on specimens unless tubercular meningitis is suspected. Considering the length of time required to culture mycobacteria, a positive report from this smear is extremely valuable.

Specimens from possible cases of fungal meningitis are Gram stained and often have an India ink preparation performed on them to detect the presence of thickly encapsulated *Cryptococcus neoformans* (Fig. 10-35). As one of the more frequently occurring complications of AIDS, cryptococcal meningitis is now commonly encountered in the clinical laboratory. Particular attention should be paid to the Gram stain for the classic starburst pattern produced by *Cryptococcus*, as this may be seen more often than a positive India ink (Fig. 10-36).²⁴

Latex agglutination tests to detect the presence of *C. neo-formans* antigen in serum and CSF provide a more sensitive method than the India ink preparation. However, immunologic testing results should be confirmed by culture and demonstration of the organisms by India ink, because false-positive reactions do occur. Interference by *rheumatoid factor* is the most common cause of false-positive reactions. Several commercial kits with pretreatment techniques are available and include incubation with dithiothreitol or pronase and boiling with ethylenediaminetetra-acetic acid. ^{21,25} An enzyme immunoassay technique has been shown to produce fewer false-positive results. ²⁶

Latex agglutination and enzyme-linked immunosorbent assay (ELISA) methods provide a rapid means for detecting and identifying microorganisms in CSF. Test kits are available to detect *Streptococcus* group B, *H. influenzae* type b, *S. pneumoniae*, *N. meningitidis* A, B, C, Y, W135, and *E. coli K1* antigens. The bacterial antigen test (BAT) does not appear to be as sensitive to detection of *N. meningitidis* as it is to the other organisms.²⁷ The BAT should be used in combination with results from the hematology and clinical chemistry laboratories for diagnosing meningitis.²⁸ The Gram stain is still the recommended method for detection of organisms.²⁹

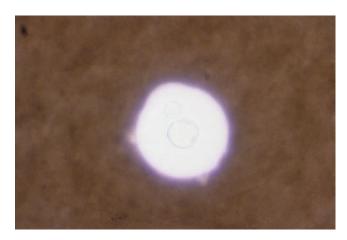


Figure 10–35 India ink preparation of *C. neoformans* (×400). Notice budding yeast form. (Courtesy of Ann K. Fulenwider, Md.)

194 CHAPTER 10 • Cerebrospinal Fluid

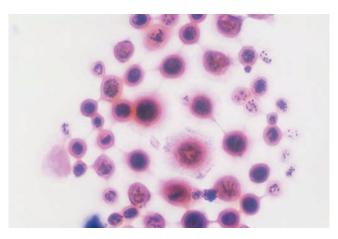


Figure 10–36 Gram stain of *C. neoformans* showing starburst pattern (×1000). (Courtesy of Ann K. Fulenwider, Md.)

Serologic Testing

In addition to the serologic procedures performed for identification of microorganisms, serologic testing of the CSF is performed to detect the presence of neurosyphilis. The use of penicillin in the early stages of syphilis has greatly reduced the number of neurosyphilis cases. Consequently, the number of requests for serologic tests for syphilis on CSF is currently low. However, detection of the antibodies associated with syphilis in the CSF still remains a necessary diagnostic procedure.

Although many different serologic tests for syphilis are available when testing blood, the procedure recommended by the Centers for Disease Control and Prevention to diagnose neurosyphilis is the Venereal Disease Research Laboratories (VDRL), even though it is not as sensitive as the fluorescent treponemal antibody-absorption (FTA-ABS) test for syphilis. If the FTA-ABS is used, care must be taken to prevent contamination with blood, because the FTA-ABS remains positive in the serum of treated cases of syphilis.

PROCEDURE



Simulated Spinal Fluid Procedure

Equipment and Reagents

- 1. Whole blood is collected the same day in EDTA. The "ideal" blood specimen for preparing SSF has a white count around 10 × 10⁹ per liter, a low platelet count, and a normal-appearing differential with at least 20% lymphocytes. To prepare 50 mL of SSF, 5 to 7 mL of blood are needed.
- 2. Hanks' balanced salt solution (102) without phenol red, sodium bicarbonate, calcium, or magnesium (Grand Island Biological Company, Grand Island, N.Y.). Dilute 1:10 with deionized water.
- 3. 30% bovine serum albumin.
- 4. Macrohematocrit tubes.

- **5**. Capillary (Pasteur type) pipettes, both standard and 9-inch lengths.
- 6. Horizontal head centrifuge. A Beckman TJ6 model (Beckman Instruments, Inc, Palo Alto, Calif.) was used for this study.

Steps

- 1. For each SSF sample, dispense 50-mL diluted balanced salt solution into a 125-mL Erlenmeyer flask. (The amount of balanced salt solution may be varied; 50 mL will make approximately 30 aliquots of SSF.)
- **2.** Centrifuge the blood in the original collection tube at 300 g for 5 min. A gray-pink buffy coat layer should be visible at the interface between the plasma and the RBCs
- 3. Aspirate off as much plasma as possible with a capillary pipette. Do not disturb the top (buffy coat) layer. Discard the plasma.
- **4.** With a 9-inch capillary pipette and a circular motion, aspirate off the remaining plasma and the entire buffy coat layer. A small amount of the RBC layer will be aspirated into the pipette at the same time. This is acceptable.
- **5**. Fill a macrohematocrit tube with this buffy coat mixture. Do not mix blood specimens from more than one source in one tube (they may agglutinate).
- **6.** Centrifuge the macrohematocrit tube at 900 g for 10 min.
- 7. Pipette off as much of the plasma as possible and discard it. If a definite white layer (platelets) is visible above the gray buffy coat, carefully remove as much of it as possible without disturbing the gray layer.
- 8. Using a clean 9-inch capillary pipette, aspirate off the buffy coat (and as little of the red cell layer as possible) and add it to the flask containing diluted balanced salt solution. Rinse the pipette several times.
- 9. Mix well and check the concentration of RBCs and WBCs by examining the SSF in a hemocytometer.
- 10. Adjust the concentration of cells as needed; add more balanced salt solution to decrease the number of RBCs and WBCs. The number of RBCs may be increased by adding more cells from the red cell layer. Since the entire buffy coat has been used, increasing the number of WBCs is not possible.
- 11. Add one drop (approximately 0.05 mL) of 30% bovine serum albumin to each 50 mL of SSF for each 30 mg/dL total protein desired.
- **12**. Mix well and dispense aliquots of approximately 1.5 mL SSF into appropriate tightly stoppered containers.

From Lofsness and Jensen, 32 with permission

The purpose of performing a test for syphilis on the CSF is to detect active cases of syphilis within the CNS. Therefore, the less sensitive VDRL procedure, the blood levels for which decrease in the later stages of syphilis, is more specific for

infection of the CNS.³⁰ The rapid plasma reagin (RPR) test is not recommended for use on CSF, because it is less sensitive and specific than the VDRL. To prevent unnecessary testing of the CSF in suspected cases of neurosyphilis, a positive serum test should be obtained using the FTA-ABS. Fluid can be frozen until serum results are available.³¹

■■● Teaching Cerebrospinal Fluid Analysis

Many of the problems that occur in the analysis of CSF are the result of inadequate training of the personnel performing the tests. This is understandable when one considers that not only is CSF difficult to collect, but also that there is often very little fluid left for student practice after the required tests have been run. Preparation of simulated fluids by adding blood cells to saline has met with limited success owing to the instability of the cells in saline and the inability to perform routine chemical analyses for glucose and protein. More satisfactory results can be achieved using the simulated spinal fluid procedure presented in this chapter, which provides the teaching laboratory with a specimen suitable for all types of cell analyses and glucose and protein determinations. The advantages of this procedure over others include the absence of bicarbonate, which may cause bubbling with acidic diluting fluids; the absence of calcium, which prevents clot formation when blood is added; stability for 48 hours under refrigeration; no distortion of cellular morphology; and the presence of glucose and protein.32

References

- 1. Hammock, M, and Milhorat, T: The cerebrospinal fluid: Current concepts of its formation. Ann Clin Lab Sci 6(1):22-28, 1976.
- 2. Edlow, JA, and Caplan, LR: Avoiding pitfalls in the diagnosis of subarachnoid hemorrhage. N Engl J Med 342:29-36, 2000.
- 3. Nagda, KK: Procoagulant activity of cerebrospinal fluid in health and disease. Indian J Med Res 74:107-110, 1981.
- Chow, G, and Schmidley, JW: Lysis of erythrocytes and leukocytes in traumatic lumbar punctures. Arch Neurol 41:1084-1085, 1984.
- 5. Seehusen, DA, Reeves, MM, and Fomin, DA: Cerebrospinal fluid analysis. Am Fam Physician 68(6):1103-1108, 2003.
- Glasser, L: Tapping the wealth of information in CSF Diagn Med 4(1):23-33, 1981.
- 7. University of Virginia Health Sciences Center: Clinical Laboratory Procedure Manual. Charlottesville, Va., 1993.
- 8. Novak, RW: Lack of validity of standard corrections for white blood cell counts of blood contaminated cerebrospinal fluid in infants. Am J Clin Pathol 82:95-97, 1984.
- Kjeldsberg, CR, and Knight, JA: Body Fluids: Laboratory Examination of Amniotic, Cerebrospinal, Seminal, Serous and Synovial Fluids: A Textbook Atlas. ASCP, Chicago, 1993.
- Abrams, J, and Schumacher, HR: Bone marrow in cerebrospinal fluid and possible confusion with malignancy. Arch Pathol Lab Med 110:366-369, 1986.
- 11. Bentz, JS: Laboratory investigation of multiple sclerosis. Lab Med 26(6):393-399, 1995.
- 12. Biou, D, et al: Cerebrospinal fluid protein concentrations in children: Age-related values in patients without disorders of the central nervous system. Clin Chem 46(3):399-403, 2000.

- 13. Fishman, RA: Cerebrospinal Fluid in Diseases of the Nervous System, 2nd ed. WB Saunders, Philadelphia, 1992.
- 14. Hershey, LA, and Trotter, JL: The use and abuse of the cerebrospinal fluid IgG profile in the adult: A practical evaluation. Ann Neurol 8(4):426-434, 1980.
- 15. Grimaldi, LME, et al: Oligoclonal IgG bands in cerebrospinal fluid and serum during asymptomatic human immunodeficiency virus infection. Ann Neurol 24:277-279, 1988.
- 16. Rouah, E, Rogers, BB, and Buffone, GJ: Transferrin analysis by immunofixation as an aid in the diagnosis of cerebrospinal fluid otorrhea. Arch Pathol Lab Med 111:756-757, 1987.
- 17. Whitaker, JN: Myelin basic protein in cerebrospinal fluid and other body fluids. Multiple Sclerosis 4(1):16-21, 1998.
- 18. Okta, M, et al: Evaluation of an enzyme immunoassay for myelin basic protein in CSF. Clin Chem 46:1326-1330, 2000.
- 19. Menkes, J: The causes of low spinal fluid sugar in bacterial meningitis: Another look. Pediatrics 44(1):1-3, 1969.
- 20. Hourani, BT, Hamlin, EM, and Reynolds, TB: Cerebrospinal fluid glutamine as a measure of hepatic encephalopathy. Arch Intern Med 127:1033-1036, 1971.
- Eng, RHK, and Person, A: Serum cryptococcal antigen determination in the presence of rheumatoid factor. J Clin Microbiol 14:700-702, 1981.
- Glasgow, AM, and Dhiensiri, K: Improved assay for spinal fluid glutamine and values for children with Reye's syndrome. Clin Chem 20(6):642-644, 1974.
- Murray, PR, and Hampton, CM: Recovery of pathogenic bacteria from cerebrospinal fluid. J Clin Microbiol 12:554-557, 1980
- Sato, Y, et al: Rapid diagnosis of cryptococcal meningitis by microscopic examination of centrifuged cerebrospinal fluid sediment. J Neurol Sci 164(1):72-75, 1999.
- Stockman, L, and Roberts, GD: Specificity of the latex test for cryptococcal antigen: A rapid simple method for eliminating interference. J Clin Microbiol 17(5):945-947, 1983.
- 26. Knight, FR: New enzyme immunoassay for detecting cryptococcal antigen. J Clin Pathol 45(9):836-837, 1992.
- Buck, GE: Nonculture methods for detection and identification of microorganisms in clinical specimens. Pediatr Clin North Am 36(1):95-100, 1989.
- Werner, V, and Kruger, RL: Value of the bacterial antigen test in the absence of CSF fluid leukocytosis. Lab Med 22(11):787-789, 1991.
- 29. Coovadia, YM, and Soliva, Z: Three latex agglutination tests compared with Gram staining for the detection of bacteria in cerebrospinal fluid. S Afr Med J 71(7):442, 1987.
- Davis, LE, and Schmitt, JW: Clinical significance of cerebrospinal fluid tests for neurosyphilis. Ann Neurol 25:50-53, 1989
- 31. Albright, RE, et al: Issues in cerebrospinal fluid management. Am J Clin Pathol 95(3):397-401, 1991.
- Lofsness, KG, and Jensen, TL: The preparation of simulated spinal fluid for teaching purposes. Am J Med Technology 49(7):493-496, 1983.

QUESTIONS STUDY

- **1.** The functions of the CSF include all of the following *except*:
 - A. Removal of metabolic wastes
 - B. Producing an ultrafiltrate of plasma
 - C. Supplying nutrients to the CNS
 - D. Protection of the brain and spinal cord

Continued

- **2.** The CSF flows through the:
 - A. Choroid plexus
 - B. Pia mater
 - C. Arachnoid space
 - D. Dura mater
- **3.** Substances present in the CSF are contolled by the:
 - A. Arachnoid granulations
 - B. Blood-brain barrier
 - C. Presence of one-way valves
 - D. Blood-CSF barrier
- 4. The CSF tube labeled 3 is sent to:
 - A. The hematology department
 - B. The chemistry department
 - C. The microbiology department
 - D. The serology department
- **5.** The CSF tube that should be refrigerated is:
 - A. Tube 1
 - B. Tube 2
 - C. Tube 3
 - D. Tube 4
- **6.** Place the appropriate letter in front of the statement that best describes CSF specimens in these two conditions:
 - A. Traumatic tap
 - B. Intracranial hemorrhage
 - Even distribution of blood in all tubes
 - Xanthochromic supernatant
 - ____Concentration of blood in tube 1 is greater than in tube 3
 - ____Specimen contains clots
- **7.** The presence of xanthochromia can be caused by all of the following *except*:
 - A. Immature liver function
 - B. RBC degradation
 - C. A recent hemorrhage
 - D. Elevated CSF protein
- **8.** A web-like pellicle in a refrigerated CSF specimen is indicative of:
 - A. Tubercular meningitis
 - B. Multiple sclerosis
 - C. Primary CNS malignancy
 - D. Viral meningitis
- 9. Given the following information, calculate the CSF WBC count: cells counted, 80; dilution, 1:10; large Neubauer squares counted, 10.
 - A. 8
 - B. 80
 - C. 800
 - D. 8000

- 10. A CSF WBC count is diluted with:
 - A. Distilled water
 - B. Normal saline
 - C. Acetic acid
 - D. Methylene blue
- 11. A total CSF cell count on a clear fluid should be:
 - A. Reported as normal
 - B. Not reported
 - C. Diluted with normal saline
 - D. Counted undiluted
- **12.** The purpose of adding albumin to CSF before cytocentrifugation is to:
 - A. Increase the cell yield
 - B. Decrease the cellular distortion
 - C. Improve the cellular staining
 - D. Both A and B
- **13**. The primary concern when pleocytosis of neutrophils and lymphocytes is found in the CSF is:
 - A. Meningitis
 - B. CNS malignancy
 - C. Multiple sclerosis
 - D. Hemorrhage
- **14.** Neutrophils with pyknotic nuclei may be mistaken for:
 - A. Lymphocytes
 - B. Nucleated RBCs
 - C. Malignant cells
 - D. Spindle-shaped cells
- **15**. The presence of which of the following cells is increased when a CNS shunt malfunctions?
 - A. Neutrophils
 - B. Macrophages
 - C. Eosinophils
 - D. Lymphocytes
- 16. Macrophages appear in the CSF following:
 - A. Hemorrhage
 - B. Repeated spinal taps
 - C. Diagnostic procedures
 - D. All of the above
- 17. Nucleated RBCs are seen in the CSF as a result of:
 - A. Elevated blood RBCs
 - B. Treatment of anemia
 - C. Severe hemorrhage
 - D. Bone marrow contamination
- **18.** Following a CNS diagnostic procedure, which of the following might be seen in the CSF?
 - A. Choroidal cells
 - B. Ependymal cells
 - C. Spindle-shaped cells
 - D. All of the above

- **19**. Hemosiderin granules and hematoidin crystals are seen in:
 - A. Lymphocytes
 - B. Macrophages
 - C. Ependymal cells
 - D. Neutrophils
- 20. Myeloblasts are seen in the CSF:
 - A. In bacterial infections
 - B. In conjunction with CNS malignancy
 - C. Following cerebral hemorrhage
 - D. As a complication of acute leukemia
- **21**. Cells resembling large and small lymphocytes with cleaved nuclei represent:
 - A. Lymphoma cells
 - B. Choroid cells
 - C. Melanoma cells
 - D. Medulloblastoma cells
- 22. The normal value of CSF protein is:
 - A. 6-8 g/dL
 - B. 15-45 g/dL
 - C. 6-8 mg/dL
 - D. 15-45 mg/dL
- **23.** CSF can be differentiated from plasma by the presence of:
 - A. Albumin
 - B Globulin
 - C. Prealbumin
 - D. Tau transferrin
- **24**. In plasma, the second most prevalent protein is IgG; in CSF, the second most prevalent protein is:
 - A. Transferrin
 - B. Prealbumin
 - C. IgA
 - D. Ceruloplasmin
- **25**. Elevated CSF protein values can be caused by all of the following *except*:
 - A. Meningitis
 - B. Multiple sclerosis
 - C. Fluid leakage
 - D. CNS malignancy
- **26**. The integrity of the blood-brain barrier is measured using the:
 - A. CSF/serum albumin index
 - B. CSF/serum globulin ratio
 - C. CSF albumin index
 - D. CSF IgG index
- **27**. Given the following results, calculate the IgG index: CSF IgG, 50 mg/dL; serum IgG, 2 gm/dL; CSF albumin, 70 mg/dL; serum albumin, 5 gm/dL.
 - A. 0.6
 - B. 6.0
 - C. 1.8
 - D. 2.8

- **28.** The CSF IgG index calculated in Study Question 27 is indicative of:
 - A. Synthesis of IgG in the CNS
 - B. Damage to the blood-brain barrier
 - C. Cerebral hemorrhage
 - D. Lymphoma infiltration
- **29**. The finding of oligoclonal bands in the CSF and not in the serum is seen with:
 - A. Multiple myeloma
 - B. CNS malignancy
 - C. Multiple sclerosis
 - D. Viral infections
- **30**. A CSF glucose of 15 mg/dL, WBC count of 5000, 90% neutrophils, and protein of 80 mg/dL is suggestive of:
 - A. Fungal meningitis
 - B. Viral meningitis
 - C. Tubercular meningitis
 - D. Bacterial meningitis
- **31.** A patient with a blood glucose of 120 mg/dL would have a normal CSF glucose of:
 - A. 20 mg/dL
 - B. 60 mg/dL
 - C. 80 mg/dL
 - D. 120 mg/dL
- **32.** CSF lactate will be more consistantly decreased in:
 - A. Bacterial meningitis
 - B. Viral meningitis
 - C. Fungal meningitis
 - D. Tubercular meningitis
- **33.** Measurement of which of the following can be replaced by CSF glutamine analysis in children with Reye syndrome?
 - A. Ammonia
 - B. Lactate
 - C. Glucose
 - D. α -ketoglutarate
- **34.** Prior to performing a Gram stain on CSF, the specimen must be:
 - A. Filtered
 - B. Warmed to 37°C
 - C. Centrifuged
 - D. Mixed
- **35.** All of the following statements are true about cryptoccocal meningitis *except*:
 - A. An India Ink preparation is positive
 - B. A starburst pattern is seen on Gram stain
 - C. The WBC count is over 2000
 - D. A confirmatory immunology test is available
- **36.** The test of choice to detect neurosyphilis is the:
 - A. RPR
 - B. VDRL
 - C. FTA
 - D. FTA-ABS

Case Studies and Clinical Situations

1. Three tubes of CSF containing evenly distributed visible blood are drawn from a 75-year-old disoriented patient and delivered to the laboratory. Initial test results are as follows:

WBC COUNT: $250~\mu L$ PROTEIN: 150~mg/dL GLUCOSE: 70~mg/dL GRAM STAIN: No organisms seen DIFFERENTIAL: Neutrophils, 68%; monocytes, 3%; lymphocytes, 28%; eosinophils, 1%

Many macrophages containing ingested RBCs

- a. What is the most probable condition indicated by these results? State two reasons for your answer.
- b. Are the elevated WBC count and protein of significance? Explain your answer.
- c. Are the percentages of the cells in the differential of any significance? Explain your answer.
- d. What two other structures besides RBCs might be contained in the macrophages?
- e. If the blood was unevenly distributed and nucleated RBCs and capillary structures were seen instead of macrophages, what would this indicate?
- 2. A patient with AIDS is hospitalized with symptoms of high fever and rigidity of the neck. Routine laboratory tests on the CSF show a WBC count of 100/μL with a predominance of lymphocytes and monocytes, glucose of 55 mg/dL (plasma: 85 mg/dL), and a protein of 70 mg/dL. The Gram stain shows a questionable starburst pattern.
 - a. What additional microscopic examination should be performed?
 - b. If the test is positive, what is the patient's diagnosis?
 - c. If the results of the test are questionable, what additional testing can be performed?
 - d. What could cause a false-positive reaction in this
 - e. If the tests named in a and c are negative, the glucose level is 35 mg/dL, and a pellicle is observed in the fluid, what additional testing should be performed?
 - f. If CSF and serum IFE was performed on this patient, what unusual findings might be present?
- **3.** A 35-year-old woman is admitted to the hospital with symptoms of intermittent blurred vision, weakness, and loss of sensation in her legs. A lumbar puncture is performed with the following results:

APPEARANCE: Colorless, clear

WBC COUNT: 35 cells/µL (90% lymphocytes) GLUCOSE: 60 mg/dL (plasma: 100 mg/dL)

PROTEIN: 60 mg/dL (serum: 8 g/dL) ALBUMIN: 40 mg/dL (serum: 6 g/dL) IGG globulin: 20 mg/dL (serum: 2 g/dL)

- a. Name and perform the calculation to determine the integrity of the patient's blood-brain barrier.
- b. Does the patient have an intact barrier?
- c. Name and perform the calculation used to determine if IgG is being synthesized within the CNS.
- d. What does this result indicate?
- e. Considering the patient's clinical symptoms and the calculation results, what diagnosis is suggested?
- f. If immunofixation electrophoresis is performed on the patient's serum and CSF, what findings would be expected?
- g. What substance in the CSF can be measured to monitor this patient?
- 4. Mary Howard, age 5, is admitted to the pediatrics ward with a temperature of 105°F, lethargy, and cervical rigidity. A lumbar spinal tap is performed, and three tubes of cloudy CSF are delivered to the laboratory. Preliminary test results are as follows:

appearance: Hazy wbc count: 800 cells/ μL

DIFFERENTIAL: 80% lymphocytes, 15% monocytes,

5% neutrophils PROTEIN: 65 mg/dL GLUCOSE: 70 mg/dL

GRAM STAIN: No organisms seen

- a. From these results, what preliminary diagnosis could the physician consider?
- b. Is the Gram stain result of particular significance? Why or why not?
- c. Are the lymphocytes of significance? Why or why not?
- d. Would a CSF lactate test be of any value for the diagnosis? Why or why not?
- **5**. State possible technical errors that could result in the following discrepancies:
 - a. An unusual number of Gram stains reported as gram-positive cocci fail to be confirmed by positive cultures.
 - b. A physician complains that CSF differentials are being reported only as polynuclear and mononuclear cells.
 - c. Bacteria observed on the cytospin differential cannot be confirmed by Gram stain or culture.
 - d. The majority of CSF specimens sent to the laboratory from the neurology clinic have glucose readings less than 50% of the corresponding blood glucose results performed in the clinic.









CHAPTER

Semen

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

- 1 State the structures involved in sperm production and their function.
- **2** Describe the four components of semen with regard to source and function.
- **3** Describe the normal appearance of semen and three abnormalities in appearance.
- **4** State two possible causes of low semen volume.
- 5 Discuss the significance of semen liquefaction and viscosity.
- 6 Calculate a sperm concentration and count when provided with the number of sperm counted, the dilution, the area of the counting chamber used, and the ejaculate volume.
- 7 Define round cells, and explain their signifi-
- **8** State the two parameters considered when evaluating sperm motility.

- **9** Describe the appearance of normal sperm, including structures and their functions.
- **10** Differentiate between routine and strict criteria for evaluation of sperm morphology.
- 11 Given an abnormal result in the routine semen analysis, determine additional tests that might be performed.
- 12 Describe the two routinely used methods for detection of antisperm antibodies.
- 13 List two methods for identifying a questionable fluid as semen.
- 14 State the World Health Organization normal values for routine and follow-up semen analysis.
- **15** Discuss the types and significance of sperm function tests.
- **16** Describe methods of quality control appropriate for the semen analysis.

KEY TERMS

acrosomal cap andrology

liquefaction semen

spermatids spermatozoa

Advances in the field of *andrology* and assisted reproductive technology (ART), and increased concern over fertility, particularly by couples choosing to have children later in life, have resulted in increased emphasis on the analysis of *semen*. Patients with abnormal results on the routine semen analysis performed in the clinical laboratory often are referred to specialized andrology laboratories for further testing to determine the need for *in vitro fertilization* (IVF). Clinical laboratory personnel may also be employed in andrology laboratories and perform both routine and specialized testing.

In addition to fertility testing, the clinical laboratory performs postvasectomy semen analysis and forensic analyses to determine the presence of semen.

Physiology

Semen is composed of four fractions that are contributed by the testes, *epididymis*, *seminal vessels*, *prostate*, and *bulbourethral glands* (Fig. 11-1). Each fraction differs in its composition, and the mixing of all four fractions during

200 CHAPTER II • Semen

ejaculation is essential for the production of a normal semen specimen (Table 11–1).

The testes contain the *seminiferous tubules*. Germ cells for the production of *spermatozoa* are located in the epithelial cells of the seminiferous tubules. Specialized Sertoli cells provide support and nutrients for the germ cells as they undergo mitosis and meiosis (spermatogenesis). When spermatogenesis is complete, the immature sperm (nonmotile) enter the epididymis. In the epididymis, the sperm mature and develop flagella. They remain stored in the epididymis until ejaculation. At that time they are propelled through the ductus deferens (vas deferens) to the ejaculatory ducts.

The ejaculatory ducts receive both the sperm from the ductus deferens and fluid from the seminal vesicles. The seminal vesicles produce the majority of the fluid present in semen (60% to 70%). The fluid contains a high concentration of fructose. Spermatozoa metabolize the fructose for the energy needed for the flagella to propel them through the female reproductive tract. In the absence of fructose, sperm do not display motility in the semen analysis.

The muscular prostate gland, located just below the bladder, surrounds the upper urethra and aids in propelling the sperm through the urethra by contractions during ejaculation. Approximately 20% to 30% of the semen volume is acidic fluid produced by the prostate gland. The acidic fluid contains high concentrations of acid phosphatase, citric acid,

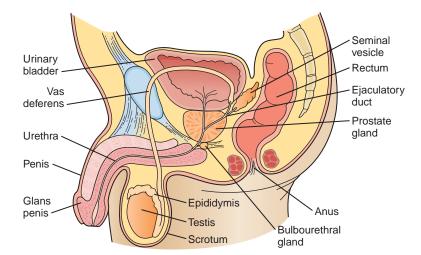
Table 11–1	Composition of Semen
Spermatozoa	5%
Seminal fluid	60%–70%
Prostate fluid	20%–30%
Bulbourethral gl	ands 5%

zinc, and proteolytic enzymes responsible for both the coagulation and *liquefaction* of the semen following ejaculation.

The bulbourethral glands, located below the prostate, contribute about 5% of the fluid volume in the form of a thick, alkaline mucus that helps to neutralize acidity from the prostate secretions and the vagina. It is important for semen to be alkaline to neutralize the vaginal acidity present as a result of normal bacterial vaginal flora. Without this neutralization, sperm motility would be diminished.

Specimen Collection

The variety in the composition of the semen fractions makes proper collection of a complete specimen essential for accurate evaluation of male fertility. The majority of sperm are contained in the first portion of the ejaculate, making com-



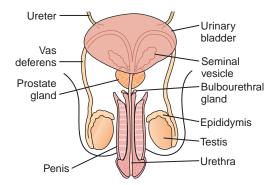


Figure II-I Diagram of the male genitalia.

Normal Values for

Semen Analysis⁴

2-5 mL

Pours in droplets

Summary of Semen Production			
Seminiferous tubules of testes	Spermatogenesis		
Epididymis	Sperm maturation		
Ductus deferens	Propel sperm to ejaculatory ducts		
Seminal vesicles	Provide nutrients for sperm and fluid		
Prostate gland	Provide enzymes and proteins for coagulation and liquefaction		
Bulbourethral glands	Add alkaline mucus to neutralize prostatic acid and vaginal acidity		

Epididymis	Sperm maturation	рН	7.2–8.0
Ductus deferens	Propel sperm to ejaculatory ducts	Sperm concentration	>20 million/mL
Seminal vesicles	Provide nutrients for sperm and fluid	Sperm count	>40 million/ejaculate
Prostate gland	Provide enzymes and proteins for	Motility	>50% within 1 hr
Trostate gland	coagulation and liquefaction	Quality	>2.0 or a, b, c in Table 11–3
Bulbourethral glands	Add alkaline mucus to neutralize prostatic acid and vaginal acidity	Morphology	>14% normal forms (strict criteria) >30% normal forms (routine criteria)
	1 0 7	Round cells	<1.0 million/mL
nlete collection essent	ial for accurate testing of both fertility	Annearance	

plete collection essential for accurate testing of both fertility and postvasectomy specimens. Patients should receive detailed instructions for specimen collection.

Specimens are collected following a period of sexual abstinence of from 2 to 3 days to not longer than 5 days. Specimens collected following prolonged abstinence tend to have higher volumes and decreased motility. When performing fertility testing, two or three samples are usually tested at 2-week intervals, with two abnormal samples considered significant. The laboratory should provide warm sterile glass or plastic containers. Whenever possible, the specimen is collected in a room provided by the laboratory. However, if this is not appropriate, the specimen should be kept at room temperature and delivered to the laboratory within 1 hour of collection. Laboratory personnel must record the time of specimen collection and specimen receipt. Specimens awaiting analysis should be kept at 37°C. Specimens should be collected by masturbation. If this is not possible, only nonlubricant-containing rubber or polyurethane condoms should be used. Ordinary condoms are not acceptable because they have spermicidal properties.

Specimen Handling

All semen specimens are potential reservoirs for HIV and hepatitis viruses, and Standard Precautions must be observed at all times during the analysis. Specimens are discarded as biohazardous waste.

Semen Analysis

The semen analysis for fertility evaluation consists of both macroscopic and microscopic examination. Parameters reported include appearance, volume, viscosity, pH, sperm concentration and count, motility, and morphology. Normal values are shown in Table 11-2.

Appearance

Table 11-2

Volume

Viscosity

Normal semen has a gray-white color, appears translucent, and has a characteristic musty odor. Increased white turbidity indicates the presence of white blood cells (WBCs) and infection within the reproductive tract. If required, specimen culturing is performed prior to continuing with the semen analysis. During the microscopic examination, WBCs must be differentiated from immature sperm (spermatids). The leukocyte esterase reagent strip test may be useful to screen for the presence of WBCs.2 Varying amounts of red coloration are associated with the presence of red blood cells (RBCs) and are abnormal. Yellow coloration may be caused by urine contamination, specimen collection following prolonged abstinence, and medications. Urine is toxic to sperm, thereby affecting the evaluation of motility.

Liquefaction

A fresh semen specimen is clotted and should liquefy within 30 to 60 minutes after collection; therefore, recording the time of collection is essential for evaluation of semen liquefaction. Analysis of the specimen cannot begin until after liquefaction has occurred. If after 2 hours the specimen has not liquified, proteolytic enzymes such as alpha-chymotrypsin may be added to allow the rest of the analysis to be performed. Failure of liquefaction to occur may be caused by a deficiency in prostatic enzymes and should be reported.

Volume

Normal semen volume ranges between 2 and 5 mL. It can be measured by pouring the specimen into a clean graduated cylinder calibrated in 0.1-mL increments. Increased volume may be seen following periods of extended abstinence. Decreased volume is more frequently associated with infertil202 CHAPTER II • Semen

ity and may indicate improper functioning of one of the semen-producing organs, primarily the seminal vesicles. Incomplete specimen collection must also be considered.

Viscosity

Specimen viscosity refers to the consistency of the fluid and may be related to specimen liquefaction. Incompletely liquefied specimens are clumped and highly viscous. The normal semen specimen should be easily drawn into a pipette and form droplets that do not appear clumped or stringy when discharged from the pipette. Normal droplets form a thin thread when released from the pipette. Droplets with threads longer that 2 centimeters are considered highly viscous. Ratings of 0 (watery) to 4 (gel-like) can be assigned to the viscosity report. Viscosity can also be reported as low, normal, and high. Increased viscosity and incomplete liquefaction impede sperm motility.

pН

The normal pH of semen is alkaline with a range of 7.2 to 8.0. Increased pH is indicative of infection within the reproductive tract. A decreased pH is associated with increased prostatic fluid. Semen for pH testing can be applied to the pH pad of a urinalysis reagent strip and the color compared with the manufacturer's chart. Dedicated pH testing paper also can be used.

Sperm Concentration/Count

Even though fertilization is accomplished by one spermatozoon, the actual number of sperm present in a semen specimen is a valid measurement of fertility. Normal values for sperm concentration are commonly listed as greater than 20 million sperm per milliliter, with concentrations between 10 and 20 million per milliliter considered borderline. The total sperm count for the ejaculate can be calculated by multiplying the sperm concentration by the specimen volume. Total sperm counts greater than 40 million per ejaculate are considered normal (20 million per milliliter \times 2 mL).

In the clinical laboratory, sperm concentration is usually performed using the Neubauer counting chamber. The sperm are counted in the same manner as cells in the cerebrospinal fluid cell count, that is, by diluting the specimen and counting the cells in the Neubauer chamber. The amount of the dilution and the number of squares counted vary among laboratories.

The most commonly used dilution is 1:20 prepared using a mechanical (positive-displacement) pipette.⁴ Dilution of the semen is essential because it immobilizes the sperm prior to counting. The traditional diluting fluid contains sodium bicarbonate and formalin, which immobilize and preserve the cells; however, good results can also be achieved using saline and distilled water.

Using the Neubauer hemocytometer, sperm are usually counted in the four corner and center squares of the large center square—similar to a manual RBC count (Fig. 11-2). Both sides of the hemocytometer are loaded and counted, and

the counts should agree within 10%. An average of the two counts is used in the calculation. If the counts do not agree, both the dilution and the counts are repeated. Counts are performed using either phase or bright-field microscopy. The addition of stain, such as crystal violet, to the diluting fluid aids in visualization when using bright-field microscopy.

Only fully developed sperm should be counted. Immature sperm and WBCs, often referred to as "round" cells, must not be included. However, their presence can be significant, and they may need to be identified and counted separately. Stain included in the diluting fluid aids in differentiation between immature sperm cells (spermatids) and leukocytes, and they can be counted in the same manner as mature sperm. Greater than 1 million leukocytes per milliliter is associated with inflammation or infection of the reproductive organs that can lead to infertility.

The presence of more than 1 million spermatids per milliliter indictes disruption of spermatogenesis. This may be caused by viral infections, exposure to toxic chemicals, and genetic disorders.

Calculation of Sperm Concentration and Sperm Count

Calculation of sperm concentration is dependent on the dilution used and the size and number of squares counted. When using the 1:20 dilution and counting the five squares (RBCs) in the large center square as described previously, the number of sperm can be multiplied by 1,000,000 (add 6 zeros) to equal the sperm concentration per milliliter. Notice that unlike blood cell counts, the sperm concentration is reported in milliliters rather than microliters. Calculation of sperm con-

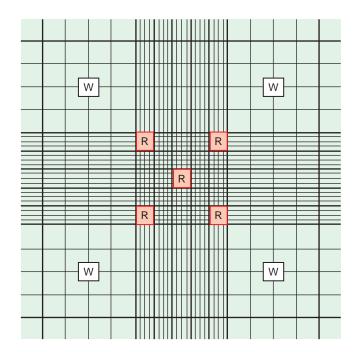


Figure 11–2 Areas of Neubauer counting chamber used for red and white blood cell counts. (W = typical WBC counting area and R = typical RBC counting area.)

centration can also be performed using the basic formula for cell counts covered in Chapter 10. Because this formula provides the number of cells per microliter, the figure must then be multiplied by 1000 to calculate the number of sperm per milliliter. The total sperm count is calculated by multiplying the number of sperm per milliliter by the specimen volume.

Examples:

1. Using a 1:20 dilution, an average of 60 sperm are counted in the five RBC counting squares on both sides of the hemocytometer. Calculate the sperm concentration per milliliter and the total sperm count in a specimen with a volume of 4 mL.

60 sperm counted
$$\times$$
 1,000,000 = $\frac{60,000,000}{\text{sperm/mL}}$
60,000,000 sperm/mL \times 4 mL = $\frac{240,000,000}{\text{sperm/ejaculate}}$

2. In a 1:20 dilution, 600 sperm are counted in two WBC counting squares. Calculate the sperm concentration per milliliter and the total sperm count in a specimen with a volume of 2 mL.

$$\frac{600 \text{ sperm counted}}{\times 20 \text{ (dilution)}} = \frac{60,000 \text{ sperm /}\mu\text{L}}{\text{(volume counted)}}$$

$$\times 0.1 \text{ }\mu\text{L (depth)}$$

$$60,000 \text{ sperm/}\mu\text{L} \times 1000 = \frac{60,000,000}{\text{sperm/mL}}$$

$$60,000,000/\text{mL} \times 2 \text{ mL} = \frac{120,000,000}{\text{sperm/ejaculate}}$$

Several methods have been developed using specially designed and disposable counting chambers that do not require dilution of the specimen. Comparison of these methods and the standard Neubauer counting chamber method showed poor correlation with the Neubauer method and also among themselves. The method recommended by the World Health Organization (WHO) is the Neubauer chamber count.⁴

Sperm Motility

The presence of sperm capable of forward, progressive movement is critical for fertility, because once presented to the cervix, the sperm must propel themselves through the cervical mucosa to the uterus, fallopian tubes, and ovum. Traditionally, clinical laboratory reporting of sperm motility has been a subjective evaluation performed by examining an undiluted specimen and determining the percentage of motile sperm and the quality of the motility.

Assessment of sperm motility should be performed on well mixed, liquefied semen within 1 hour of specimen collection. The practice of examining sperm motility at timed intervals over an extended period has been shown to serve no useful purpose. 5 To provide continuity in reporting, laboratories should place a consistent amount of semen under the same size coverslip, such as 10 μL under a 22 \times 22 mm coverslip. The percentage of sperm showing actual forward movement can then be estimated after evaluating approximately 20 high-power fields. Motility is evaluated by both speed and direction. Grading can be done using a scale of 0 to 4, with 4 indicating rapid, straight-line movement and 0 indicating no movement (Table 11–3). A minimum motility of 50% with a rating of 2.0 after 1 hour is considered normal. 1

The WHO uses a rating scale of a, b, c, d (see Table 11–3). Interpretation states that within 1 hour, 50% or more sperm should be motile in categories a, b, and c, or 25% or more should show progressive motility (a and b).⁴

The presence of a high percentage of immobile sperm and clumps of sperm requires further evaluation to determine sperm viability or the presence of sperm agglutinins.

In recent years, instrumentation capable of performing computer-assisted semen analysis (CASA) has been developed. CASA provides objective determination of both sperm velocity and trajectory (direction of motion). Sperm concentration and morphology are also included in the analysis. Currently, CASA instrumentation is found primarily in laboratories that specialize in andrology and perform a high volume of semen analysis.

Sperm Morphology

Just as the presence of a normal number of sperm that are nonmotile produces infertility, the presence of sperm that are morphologically incapable of fertilization also results in infertility.

Sperm morphology is evaluated with respect to the structure of the head, neckpiece, midpiece, and tail. Abnormalities in head morphology are associated with poor ovum penetration, whereas neckpiece, midpiece, and tail abnormalities affect motility.

The normal sperm has an oval-shaped head approximately 5 μ m long and 3 μ m wide and a long, flagellar tail approximately 45 μ m long (Fig. 11-3). Critical to ovum penetration is the enzyme-containing *acrosomal cap* located at the tip of the head. The acrosomal cap should encompass approximately half of the head and covers appproximately two

Table	211–3	Sperm Motility Grading
	Grade	WHO Criteria
4.0	а	Rapid, straight-line motility
3.0	b	Slower speed, some lateral movement
2.0	b	Slow forward progression, noticeable lateral movement
1.0	С	No forward progression
0	d	No movement

204 CHAPTER II • Semen

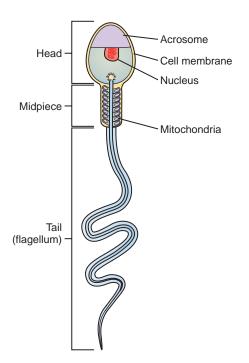


Figure II-3 Normal spermatozoa structure.

thirds of the sperm nucleus.⁴ The neckpiece attaches the head to the tail and the midpiece. The midpiece is the thickest part of the tail because it is surrounded by a mitochondrial sheath that produces the energy required by the tail for motility.

Sperm morphology is evaluated from a thinly smeared, stained slide under oil immersion. Staining can be performed using Wright's, Giemsa, or Papanicolaou stain and is a matter of laboratory preference. Air-dried slides are stable for 24 hours. At least 200 sperm should be evaluated and the percentage of abnormal sperm reported. Routinely identified abnormalities in head structure include double heads, giant and amorphous heads, pinheads, tapered heads, and constricted heads (Figs. 11-4 and 11-5). Abnormal sperm tails are frequently doubled, coiled, or bent (Figs. 11-6 and 11-7).

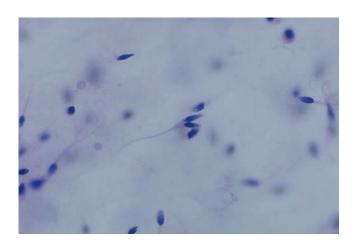


Figure 11–4 Spermatozoa with double head, hematoxylineosin (×1000).

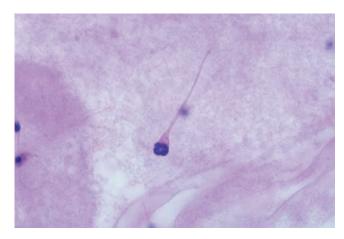


Figure 11–5 Spermatozoa with amorphous head, hematoxylineosin (×1000).

An abnormally long neckpiece may cause the sperm head to bend backward and interfere with motility (Fig. 11-8).

Additional parameters in the evaluation of sperm morphology include measurement of head, neck, and tail size, size of the acrosome, and the presence of vacuoles. Inclusion of these parameters is referred to as Kruger's strict criteria. Performance of strict criteria evaluation requires the use of a stage micrometer or morphometry. At present, evaluation of sperm morphology using strict criteria is not routinely performed in the clinical laboratory but is recommended by the WHO. Strict criteria evaluation is an intregal part of assisted reproduction evaluations.

Normal values for sperm morphology depend on the method of evaluation used and vary from greater than 30% normal forms when using routine criteria to greater than 14% normal forms when using strict criteria.⁴

Calculation of Round Cells

Differentiation and enumeration of round cells (immature sperm and leukocytes) can also be made during the morphol-



Figure 11–6 Spermatozoa with double tail, hematoxylineosin (×1000).

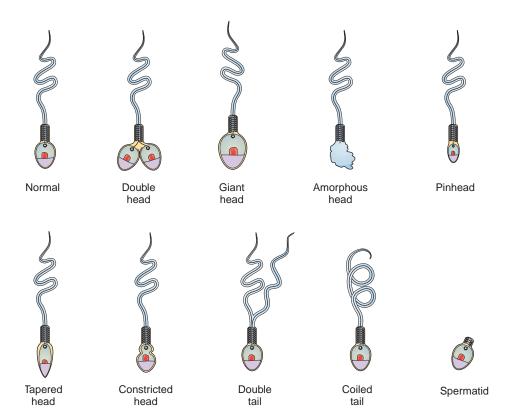


Figure 11–7 Abnormalities of sperm heads and tails are illustrated.

ogy examination (Fig. 11-9). By counting the number of spermatids or leukocytes seen in conjunction with 100 mature sperm, the amount per milliliter can be calculated using the formula:

$$C = \frac{N \times S}{100}$$

N equals the number of spermatids or neutrophils counted per 100 mature sperm, and S equals the sperm concentration in millions per milliliter. This method can be used when counting cannot be performed during the hemocytometer count and to verify counts performed by hemocytometer.

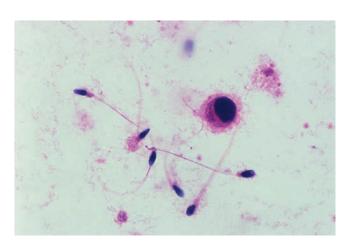


Figure 11–8 Spermatoza with bent neck and spermatid, hematoxylin-eosin (×1000).

Additional Testing

Should abnormalities be discovered in any of these routine parameters, additional tests may be requested (Table 11–4). The most common are tests for sperm viability, seminal fluid fructose level, sperm agglutinins, and microbial infection.

Sperm Viability

Decreased sperm viability may be suspected when a specimen has a normal sperm concentration with markedly decreased motility. Viability is evaluated by mixing the specimen with an eosin-nigrosin stain, preparing a smear, and counting the

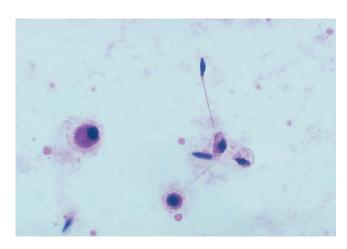


Figure 11–9 Immature spermatozoa, hematoxylin-eosin (x1000)

206 CHAPTER II • Semen

Table 11–4 Additional Testing for Abnormal Semen Analysis				
Abnormal Result	Possible Abnormality	Test		
Decreased motility with normal count	Viability	Eosin-nigrosin stain		
Decreased count	Lack of seminal vesicle support medium	Fructose level		
Decreased motility with clumping	Male antisperm antibodies	Mixed aggluti- nation reaction and immuno- bead tests Sperm aggluti- nation with male serum		
Normal analysis with continued infertility	Female anti- sperm antibodies	Sperm aggluti- nation with female serum		

number of dead cells in 100 sperm. Living cells are not infiltrated by the dye and remain a bluish white color, whereas dead cells stain red against the purple background (Fig. 11-10). Normal viability requires 75% living cells and should correspond to the previously evaluated motility.

Seminal Fluid Fructose

Low sperm concentration may be caused by lack of the support medium produced in the seminal vesicles. This can be indicated by a low to absent fructose level in the semen. Specimens can be screened for the presence of fructose using the

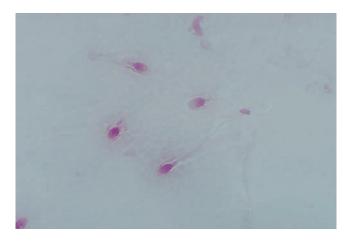


Figure 11-10 Nonviable spermatozoa demonstrated by the eosin-nigrosin stain ($\times 1000$).

PROCEDURE



Seminal Fructose Screening Test⁵

- Prepare reagent.
 (50 mg resorcinol in 33 mL concentrated HCl diluted to 100 mL with water).
- 2. Mix 1 mL semen with 9 mL reagent.
- 3 Roil
- 4. Observe for orange-red color.

resorcinol test that produces an orange color when fructose is present.

A normal quantitative level of fructose is equal to or greater than 13 μmol per ejaculate. This can be determined using spectrophotometric methods. Specimens for fructose levels should be tested within 2 hours or frozen to prevent fructolysis.

Antisperm Antibodies

Antisperm antibodies can be present in both men and women. They may be detected in semen, cervical mucosa, or serum and are considered a possible cause of infertility. It is not unusual for both partners to demonstrate antibodies, although male antisperm antibodies are more frequently encountered.

Under normal conditions, the blood-testes barrier separates sperm from the male immune system. When this barrier is disrupted, as can occur following surgery, *vasectomy* reversal (*vasovasostomy*), trauma, and infection, the antigens on the sperm produce an immune response that damages the sperm. The damaged sperm may cause the production of antibodies in the female partner.⁸

The presence of antibodies in a male subject can be suspected when clumps of sperm are observed during a routine semen analysis. The presence of antisperm antibodies in a female subject results in a normal semen analysis accompanied by continued infertility. The presence of antisperm antibodies in women may be demonstrated by mixing the semen with the female cervical mucosa or serum and observing for agglutination. A variety of immunoassay kits are available for both semen and serum testing.

Two frequently used tests to detect the presence of antibody-coated sperm are the mixed agglutination reaction (MAR) test and the immunobead test. The MAR test is a screening procedure used primarily to detect the presence of immunoglobulin G (IgG) antibodies. The semen sample containing motile sperm is incubated with IgG antihuman globulin (AHG) and a suspension of latex particles or treated RBCs coated with IgG. The bivalent AHG binds simultaneously to both the antibody on the sperm and the antibody on the latex particles or RBCs, forming microscopically visible clumps of sperm and particles or cells. Less than

10% of the motile sperm attached to the particles is considered normal.

The immunobead test is a more specific procedure in that it can be used to detect the presence of IgG, IgM, and IgA antibodies and demonstrates what area of the sperm (head, neckpiece, midpiece, or tail) the autoantibodies are affecting. Head-directed antibodies can interfere with penetration into the cervical mucosa or ovum, whereas tail-directed antibodies affect movement through the cervical mucosa. In the immunobead test, sperm are mixed with polyacrylamide beads known to be coated with either anti-IgG, anti-IgM, or anti-IgA. Microscopic examination of the sperm shows the beads attached to sperm at particular areas. Depending on the type of beads used, the test could be reported as "IgM tail antibodies," "IgG head antibodies," and so forth. The presence of beads on less than 20% of the sperm is considered normal.

Microbial and Chemical Testing

The presence of more than 1 million leukocytes per millimeter indicates infection within the reproductive system, frequently the prostate. Routine aerobic and anaerobic cultures and tests for *Chlamydia trachomatis*, *Mycoplasma hominis*, and *Ureaplasma urealyticum* are most frequently performed.

Additional chemical testing performed on semen may include determination of the levels of neutral α -glucosidase, zinc, citric acid, and prostatic acid phosphatase. Just as decreased fructose levels are associated with a lack of seminal fluid, decreased neutral α -glucosidase suggests a disorder of the epididymis. Decreased zinc, citrate, and acid phosphatase indicate a lack of prostatic fluid (Table 11–5).

On certain occasions, the laboratory may be called on to determine whether semen is actually present in a specimen. A primary example is in cases of alleged rape. Microscopically examining the specimen for the presence of sperm may be possible, with the best results being obtained by enhancing the specimen with xylene and examining under phase microscopy. Deminal fluid contains a high concentration of prostatic acid phosphatase, therefore the detection of this enzyme can aid in determining the presence of semen in a specimen. A more specific method is the detection of seminal glycoprotein p30. Turther, information can often be obtained by performing ABO blood grouping and DNA analysis on the specimen.

Table 11-5 Normal Semen Chemical Values 15			
Neutral α-glucosidase	≥20 mU/ejaculate		
Zinc	≥2.4 µmol/ejaculate		
Citric acid	≥52 µmol/ejaculate		
Acid phosphatase	≥200 Units/ejaculate		

Postvasectomy Semen Analysis

Postvasectomy semen analysis is a much less involved procedure when compared with the infertility analysis, inasmuch as the only concern is the presence or absence of spermatozoa. The length of time required for complete sterilization can vary greatly among patients and depends on both time and number of ejaculations. Therefore, finding viable sperm in a postvasectomy patient is not uncommon, and care should be taken not to overlook even a single sperm. Specimens are routinely tested at monthly intervals, beginning at 2 months postvasectomy and continuing until two consecutive monthly specimens show no spermatozoa.

Recommended testing includes examination of a wet preparation using phase microscopy for the presence of motile and nonmotile sperm. A negative wet preparation is followed by centrifugation of the specimen for 10 minutes and examination of the sediment.⁵

Sperm Function Tests

Advances in assisted reproduction and IVF have resulted in a need for more sophisticated semen analysis to assess not only the characteristics of sperm but also the functional ability. The tests are most commonly performed in specialized andrology laboratories and include the hamster egg penetration assay, cervical mucus penetration test, hypo-osmotic swelling test, and the in vitro acrosome reaction (Table 11–6).¹²

Semen Analysis Quality Control

Traditionally, semen routine analysis has been subject to very little quality control.¹³ This has resulted from a lack of appropriate control materials and the subjectivity of the motility and morphology analyses. The analysis is rated as a high complexity test under the Clinical Laboratory Improvement Amendments, and testing personnel standards must be observed.

Increased interest in fertility testing has promoted the development of quality control materials and in-depth training programs. The standardized procedures developed by the WHO have provided a basis for laboratory testing and report-

Table 11-6	Sperm Function Tests
Test	Description
Hamster egg penetration	Sperm are incubated with species- nonspecific hamster eggs and penetra- tion is observed microscopically
Cervical mucus penetration	Observation of sperm penetration ability of partner's midcycle cervical mucus
Hypo-osmotic swelling	Sperm exposed to low-sodium concentrations are evaluated for membrane integrity and sperm viability
In vitro acrosome reaction	Evaluation of the acrosome to produce enzymes essential for ovum penetration

208 CHAPTER II • Semen

ing. The use of CASA has aided in reducing the subjectivity of the analysis. However, even computerized, the analysis has been shown to vary among operators.¹⁴

Laboratories can now participate in proficiency testing programs offered by the College of American Pathologists and the American Association of Bioanalysts (AAB) that include sperm concentration, viability, and morphology. Commercial quality control materials and training aids are available and should be incorporated into laboratory protocols.

References

- Sarhar, S, and Henry, JB: Andrology laboratory and fertility assessment. In Henry, JB (ed): Clinical Diagnosis and Management by Laboratory Methods. WB Saunders, Philadelphia, 1996.
- 2. Lopez, A, et al: Suitability of solid-phase chemistry for quantification of leukocytes in cerebrospinal, seminal and peritoneal fluid. Clin Chem 33(8):1475-1476, 1987.
- 3. Overstreet, JW, and Katz, DF: Semen analysis. Urol Clin North Am 14(3):441-449, 1987.
- World Health Organization: WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Interaction. Cambridge University Press, London, 1999.
- Sampson, JH, and Alexander, NJ: Semen analysis: A laboratory approach. Lab Med 13(4):218-223, 1982.
- Kruger, T, et al: Predictive value of sperm morphology in IVF. Fertil Steril 112-117, 1988.
- 7. Harr, R: Characterization of spermatozoa by planar morphometry. Clin Lab Sci 10(4):190-196, 1997.
- Cearlock, DM: Autoimmune antispermatozoa antibodies in men: Clinical detection and role in infertility. Clin Lab Sci 2(3):165-168, 1989.
- 9. Marshburn, PB, and Kutteh, WH: The role of antisperm antibodies in infertility. Fertil Steril 61:799-811, 1994.
- 10. Fraysier, HD: A rapid screening technique for the detection of spermatozoa. J Forensic Sci 32(2):527-528, 1987.
- 11. Graves, HC, Sensabaugh, CF, and Blake, ET: Postcoital detection of a male-specific semen protein, application to the investigation of rape. N Engl J Med 312(6):338-343, 1985.
- 12. Yablonsky, T: Male fertility testing. Lab Med 27(6):378-383, 1996
- 13. Baker, DJ, et al: Semen evaluations in the clinical laboratory. Lab Med 25(8):509-514, 1994.
- 14. Krause, W, and Viethen, G: Quality assessment of computerassisted semen analysis (CASA) in the andrology laboratory. Andrologia 31(3):125-129, 1999.
- 15. Tomlinson, M, et al: One-step disposable chambers for sperm concentration and motility assessment: How do they compare with the WHO-recommended methods? Hum Reprod 16(1): 121-124, 2001.

QUESTIONS

- 1. Maturation of spermatozoa takes place in the:
 - A. Sertoli cells
 - B. Seminiferous tubules
 - C. Epididymis
 - D. Seminal vesicles

- **2.** Enzymes for the coagulation and liquefaction of semen are produced by the:
 - A. Seminal vesicles
 - B. Bulbourethral glands
 - C. Ductus deferens
 - D. Prostate gland
- 3. The major component of seminal fluid is:
 - A. Glucose
 - B. Fructose
 - C. Acid phosphatase
 - D. Citric acid
- **4.** If the first portion of a semen specimen is not collected, the semen analysis will have an abnormal:
 - A. ph
 - B. Viscosity
 - C. Sperm concentration
 - D. Sperm motility
- **5.** Failure of laboratory personnel to document the time a semen sample is collected primarily affects the interpretation of semen:
 - A. Appearance
 - B. Volume
 - C. ph
 - D. Viscosity
- **6.** Liquefaction of a semen specimen should take place within:
 - A. 1 hour
 - B. 2 hours
 - C. 3 hours
 - D. 4 hours
- 7. A semen specimen delivered to the laboratory in a condom has a normal sperm count and markedly decreased sperm motility. This is indicative of:
 - A. Decreased fructose
 - B. Antispermicide in the condom
 - C. Increased semen viscosity
 - D. Increased semen alkalinity
- 8. An increased semen ph may be caused by:
 - A. Prostatic infection
 - B. Decreased prostatic secretions
 - C. Decreased bulbourethral gland secretions
 - D. All of the above
- **9.** Proteolytic enzymes may be added to semen specimens to:
 - A. Increase the viscosity
 - B. Dilute the specimen
 - C. Decrease the viscosity
 - D. Neutralize the specimen

- 10. The normal sperm concentration is:
 - A. Below 20 million per microliter
 - B. Above 20 million per milliliter
 - C. Below 20 million per milliliter
 - D. Above 20 million per microliter
- 11. Given the following information, calculate the sperm concentration: dilution, 1:20; sperm counted in five RBC squares on each side of the hemocytometer, 80 and 86; volume, 3 mL.
 - A. 80 million per milliliter
 - B. 83 million per milliliter
 - C. 86 million per milliliter
 - D. 169 million per microliter
- **12.** Using the above information, calculate the sperm concentration when 80 sperm are counted in 1 WBC square and 86 sperm are counted in another WBC square.
 - A. 83 million per milliliter
 - B. 166 million per ejaculate
 - C. 16.6 million per milliliter
 - D. 50 million per ejaculate
- **13**. The primary reason to dilute a semen specimen before performing a sperm concentration is to:
 - A. Immobilize the sperm
 - B. Facilitate the chamber count
 - C. Decrease the viscosity
 - D. Stain the sperm
- 14. When performing a sperm concentration, 60 sperm are counted in the RBC squares on one side of the hemocytometer and 90 sperm are counted in the RBC squares on the other side. The specimen is diluted 1:20. The:
 - A. Specimen should be rediluted and counted
 - B. Sperm count is 75 million per milliliter
 - C. Sperm count is greater than 5 million per milliliter
 - D. Sperm concentation is abnormal
- 15. Sperm motility evaluations are performed:
 - A. Immediately after the specimen is collected
 - B. Within 1–2 hours of collection
 - C. After 3 hours of incubation
 - D. At 6-hour intervals for one day
- **16.** Sperm motility is evaluated on the basis of:
 - A. Speed
 - B. Direction
 - C. Tail movement
 - D. Both A and B
- **17**. The percentage of sperm showing average motility that is considered normal is:
 - A. 25%
 - B. 50%
 - C. 60%
 - D. 75%

- **18.** All of the following are grading criteria for sperm motility *except*:
 - A. Rapid straight-line movement
 - B. Rapid lateral movement
 - C. No forward progression
 - D. No movement
- 19. The purpose of the acrosomal cap is:
 - A. Ovum penetration
 - B. Protection of the nucleus
 - C. Energy for tail movement
 - D. Protection of the neckpiece
- **20**. The sperm part containing a mitochondrial sheath is the:
 - A. Head
 - B. Neckpiece
 - C. Midpiece
 - D. Tail
- **21.** All of the following are associated with sperm motility *except* the:
 - A. Head
 - B. Neckpiece
 - C. Midpiece
 - D. Tail
- 22. The morphologic shape of a normal sperm head is:
 - A. Round
 - B. Tapered
 - C. Oval
 - D. Amorphous
- **23.** Normal sperm morphology when using the WHO criteria is:
 - A. >30% normal forms
 - B. <30% normal forms
 - C. >15% abnormal forms
 - D. <15% normal forms
- **24**. Additional parameters measured by Kruger strict morphology include all of the following *except*:
 - A. Viability
 - B. Presence of vacuoles
 - C. Acrosome size
 - D. Tail length
- **25.** Round cells that are of concern and may be included in sperm counts and morphology analysis are:
 - A. Leukocytes
 - B. Spermatids
 - C. RBCs
 - D. Both A and B
- **26**. If 5 round cells per 100 sperm are counted in a sperm morphology smear and the sperm concentation is 30 million, the concentration of round cells is:
 - A. 150,000
 - B. 1.5 million
 - C. 300,000
 - D. 15 million

210 CHAPTER II • Semen

Continued

- 27. Following an abnormal sperm motility test with a normal sperm count, what additional test might be ordered?
 - A. Fructose level
 - B. Zinc level
 - C. Mar test
 - D. Eosin-nigrosin stain
- **28**. Follow-up testing for a low sperm concentration would include testing for:
 - A. Antisperm antibodies
 - B. Seminal fluid fructose
 - C. Sperm viability
 - D. Prostatic acid phosphatase
- 29. The immunobead test for antisperm antibodies:
 - A. Detects the presence of male antibodies
 - B. Determines the presence of IgG, IgM, and IgA antibodies
 - C. Determines the location of antisperm antibodies
 - D. All of the above
- 30. Measurement of α -glucosidase is performed to detect a disorder of the:
 - A. Seminiferous tubules
 - B. Epididymis
 - C. Prostate gland
 - D. Bulbourethral glands
- **31**. A specimen delivered to the laboratory with a request for prostatic acid phosphatase and glycoprotein p30 was collected to determine:
 - A. Prostatic infection
 - B. Presence of antisperm antibodies
 - C. A possible rape
 - D. Successful vasectomy
- **32.** Following a negative postvasectomy wet preparation, the specimen should be:
 - A. Centrifuged and reexamined
 - B. Stained and reexamined
 - C. Reported as no sperm seen
 - D. Both A and B
- **33.** Standardization of procedures and reference values for semen analysis is primarily provided by the:
 - A. Manufacturers of instrumentation
 - B. WHO
 - C. Manufacturers of control samples
 - D. Clinical laboratory improvement amendments

Case Studies and Clinical Situations

1. A repeat semen analysis for fertility testing is reported as follows:

VOLUME: 3.5 mL SPERM CONCENTRATION:

6 million/mL

VISCOSITY: Normal SPERM MOTILITY: 30%—

grade 1.0

ph: 7.5 Morphology: <30% nor-

mal forms—30 spermatids/100 sperm

The results correspond with the first analysis.

- a. List three abnormal parameters.
- b. What is the sperm count? Is this normal?
- c. What is the spermatid count? Is this normal?
- d. Could the sperm concentration and the spermatid count be related to the infertility? Explain your answer.
- **2.** A semen analysis on a postvasovasectomy patient has a normal sperm concentration; however, motility is decreased, and clumping is observed on the wet preparation.
 - a. Explain the possible connection between these observations and the patient's recent surgery.
 - b. What tests could be performed to further evaluate the patient's infertility?
 - c. Briefly explain the different interpretations offered by these two tests.
 - d. State three ways in which a positive result on these tests could be affecting male fertility.
- **3.** A yellow-colored semen specimen is received in the laboratory. The analysis is normal except for decreased sperm motility. Explain the possible connection between the two abnormal findings.
- 4. Abnormal results of a semen analysis are volume = 1.0 mL and sperm concentration = 1 million per milliliter. State a nonpathologic cause of these abnormal results.
- **5**. A semen specimen with normal initial appearance fails to liquefy after 60 minutes.
 - a. Would a specimen pH of 9.0 be consistent with this observation? Why or why not?
 - b. State three chemical tests that would be of value in this analysis.
 - c. How does this abnormality affect fertility?
- **6.** A specimen is delivered to the laboratory with a request to determine if semen is present.
 - a. What two chemical tests could be performed on the specimen?
 - b. What additional examination could be performed on the specimen?











Synovial Fluid

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

- 1 Describe the formation and function of synovial fluid.
- **2** Relate laboratory test results to the four common classifications of joint disorders.
- **3** State the five most diagnostic tests performed on synovial fluid.
- **4** Determine the appropriate collection tubes for requested laboratory tests on synovial fluid.
- 5 Describe the appearance of synovial fluid in normal and abnormal states.
- **6** Discuss the normal and abnormal cellular composition of synovial fluid.

- 7 List and describe six crystals found in synovial fluid
- 8 Explain the differentiation of monosodium urate and calcium pyrophosphate crystals using polarized and compensated polarized light.
- **9** State the clinical significance of glucose and lactate tests on synovial fluid.
- **10** List four genera of bacteria most frequently found in synovial fluid.
- **11** Describe the relationship of serologic testing of serum to joint disorders.

KEY TERMS

arthritis arthrocentesis

hyaluronic acid synovial fluid synoviocyte

■■● Physiology

Synovial fluid, often referred to as "joint fluid," is a viscous liquid found in the cavities of the movable joints (diarthroses) or synovial joints. As shown in Figure 12-1, the bones in the synovial joints are lined with smooth articular cartilage and separated by a cavity containing the synovial fluid. The joint is enclosed in a fibrous joint capsule lined by the synovial membrane. The synovial membrane contains specialized cells called **synoviocytes**. The smooth articular cartilage and synovial fluid reduce friction between the bones during joint movement. In addition to providing lubrication in the joints, synovial fluid provides nutrients to the articular cartilage and lessens the shock of joint compression that occurs during activities such as walking and jogging.

Synovial fluid is formed as an ultrafiltrate of plasma across the synovial membrane. The filtration is nonselective

except for the exclusion of high molecular weight proteins. Therefore, the majority of the chemical constituents, although seldom of clinical significance, have concentrations similar to plasma values. They do, however, provide nutrients for the vascular- deficient cartilage. The synoviocytes secrete a mucopolysaccharide containing hyaluronic acid and a small amount of protein (approximately one fourth of the plasma concentration) into the fluid. The large hyaluronate molecules contribute the noticeable viscosity to the synovial fluid. Damage to the articular membranes produces pain and stiffness in the joints, collectively referred to as arthritis. Laboratory results of synovial fluid analysis can be used to determine the pathologic origin of arthritis. The beneficial tests most frequently performed on synovial fluid are the white blood cell (WBC) count, differential, Gram stain, culture, and crystal examination.1 Normal values are shown in Table 12-1.2

212 CHAPTER I2 • Synovial Fluid

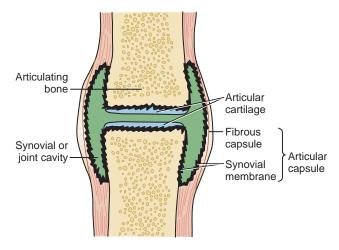


Figure 12-1 Diagram of a synovial joint.

A variety of conditions including infection, inflammation, metabolic disorders, trauma, physical stress, and advanced age are associated with arthritis. Disorders are frequently classified into four groups, as shown in Table 12–2. Some overlap of test results among the groups may occur (Table 12–3); the patient's clinical history must also be considered when assigning a category.

Specimen Collection and Handling

Synovial fluid is collected by needle aspiration called *arthrocentesis*. The amount of fluid present varies with the size of the joint and the extent of fluid buildup in the joint. For example, the normal amount of fluid in the adult knee cavity is less than 3.5 mL, but can increase to greater than 25 mL with inflammation. In some instances, only a few drops of fluid are obtained, but these can still be used for microscopic analysis or culturing. The volume of fluid collected should be recorded.

Normal synovial fluid does not clot; however, fluid from a diseased joint may contain fibrinogen and will clot. There-

	Normal Synovial Fluid Values ²
Volume	<3.5 mL
Color	Colorless to pale yellow
Clarity	Clear
Viscosity	Able to form a string 4-6 cm long
Leukocyte count	<200 cells/μL
Neutrophils	<25% of the differential
Crystals	None present
Glucose:plasma difference	<10 mg/dL lower than the blood glucose
Total protein	<3 g/dL

Pat	ssification and thologic Significance Joint Disorders
Group Classification	Pathologic Significance
1. Noninflammatory	Degenerative joint disorders, osteoarthritis
2. Inflammatory	Immunologic disorders, rheumatoid arthritis, lupus erythematosus, scleroderma, polymyositis, anklylosing spondylitis, rheumatic fever, and Lyme arthritis Crystal-induced gout and pseudogout
3. Septic	Microbial infection
4. Hemorrhagic	Traumatic injury, tumors, hemophilia, other coagulation disorders Anticoagulant overdose

Laboratory Findings in Joint Disorders^{2,6}

Table 12–3

in joint Disorders-"			
Group Classification	Laboratory Findings		
1. Noninflammatory	Clear, yellow fluid Good viscosity WBCs <1000 µL Neutrophils <30% Normal glucose (similar to blood glucose)		
2. Inflammatory (immunologic origin)	Cloudy, yellow fluid Poor viscosity WBCs 2000–75,000 µL Neutrophils >50% Decreased glucose level Possible autoantibodies present		
(crystal-induced origin)	Cloudy or milky fluid Low viscosity WBCs up to 100,000 µL Neutrophils <70% Decreased glucose level Crystals present		
3. Septic	Cloudy, yellow-green fluid Variable viscosity WBCs 50,000–100,000 µL Neutrophils >75% Decreased glucose level Positive culture and Gram stain		
4. Hemorrhagic	Cloudy, red fluid Low viscosity WBCs equal to blood Neutrophils equal to blood Normal glucose level		

fore, fluid is often collected in a syringe that has been moistened with heparin. When sufficient fluid is collected, it should be distributed into the following tubes based on the required tests:

- A sterile heparinized tube for Gram stain and culture
- A heparin or ethylenediaminetetraacetic acid (EDTA) tube for cell counts
- A nonanticoagulated tube for other tests
- A sodium fluoride tube for glucose analysis

Powdered anticoagulants should not be used because they may produce artifacts that interfere with crystal analysis. The nonanticoagulated tube for other tests must be centrifuged and separated to prevent cellular elements from interfering with chemical and serologic analyses. Ideally, all testing should be done as soon as possible to prevent cellular lysis and possible changes in crystals.

■■● Color and Clarity

A report of the gross appearance is an essential part of the synovial fluid analysis. Normal synovial fluid appears colorless to pale yellow. The word "synovial" comes from the Latin word for egg. Normal viscous synovial fluid resembles egg white. The color becomes a deeper yellow in the presence of noninflammatory and inflammatory *effusions* and may have a greenish tinge with bacterial infection. As with cerebrospinal fluid, in synovial fluid the presence of blood from a hemorrhagic arthritis must be distinguished from blood from a traumatic aspiration. This is accomplished primarily by observing the uneven distribution of blood in the specimens obtained from a traumatic aspiration.

Turbidity is frequently associated with the presence of WBCs; however, synovial cell debris and fibrin also produce turbidity. The fluid may appear milky when crystals are present.

■ ■ • Viscosity

Viscosity of the synovial fluid comes from the polymerization of the hyaluronic acid and is essential for the proper lubrication of the joints. Arthritis affects both the production of hyaluronate and its ability to polymerize, thus decreasing the viscosity of the fluid. Several methods are available to measure the viscosity of the fluid, the simplest being to observe the ability of the fluid to form a string from the tip of a syringe, and can be done at the bedside. A string that measures 4 to 6 cm is considered normal.

Measurement of the amount of hyaluronate polymerization can be performed using a Ropes, or *mucin* clot, test. When added to a solution of 2% to 5% acetic acid, normal synovial fluid forms a solid clot surrounded by clear fluid. As the ability of the hyaluronate to polymerize decreases, the clot becomes less firm, and the surrounding fluid increases in turbidity. The mucin clot test is reported in terms of good (solid clot), fair (soft clot), low (friable clot), and poor (no clot). The mucin clot test is not routinely performed, because all forms

of arthritis decrease viscosity and little diagnostic information is obtained. Formation of a mucin clot following the addition of acetic acid can be used to identify a questionable fluid as synovial fluid.

Cell Counts

The total leukocyte count is the most frequently performed cell count on synovial fluid. Red blood cell (RBC) counts are seldom requested. To prevent cellular disintegration, counts should be performed as soon as possible or the specimen should be refrigerated. Very viscous fluid may need to be pretreated by adding a pinch of hyaluronidase to 0.5 mL of fluid or one drop of 0.05% hyaluronidase in phosphate buffer per milliliter of fluid and incubating at 37°C for 5 minutes.³

Manual counts on thoroughly mixed specimens are done using the Neubauer counting chamber in the same manner as cerebrospinal fluid counts. Clear fluids can usually be counted undiluted, but dilutions are necessary when fluids are turbid or bloody. Dilutions can be made using the procedure presented in Chapter 10; however, traditional WBC diluting fluid cannot be used because it contains acetic acid that causes the formation of mucin clots. Normal saline can be used as a diluent. If it is necessary to lyse the RBCs, hypotonic saline (0.3%) or saline that contains saponin is a suitable diluent. Methylene blue added to the normal saline stains the WBC nuclei, permitting separation of the RBCs and WBCs during counts performed on mixed specimens. Automated cell counters can be used for synovial fluid counts; however, highly viscous fluid may block the apertures, and the presence of debris and tissue cells may falsely elevate counts. As described previously, incubation of the fluid with hyaluronidase decreases the specimen viscosity. Analysis of scattergrams can aid in the detection of tissue cells and debris. Properly controlled automated counts provide higher precision than manual counts.4,5

WBC counts less than 200 cells/ μ L areL considered normal and may reach 100,000 cells/ μ L or higher in severe infections. There is, however, considerable overlap of elevated leukocyte counts between septic and inflammatory forms of arthritis. Pathogenicity of the infecting organisms also produces varying results in septic arthritis, as does antibiotic administration.

Differential Count

Differential counts should be performed on cytocentrifuged preparations or on thinly smeared slides. Fluid should be incubated with hyaluronidase prior to slide preparation. Mononuclear cells, including monocytes, macrophages, and synovial tissue cells, are the primary cells seen in normal synovial fluid. Neutrophils should account for less than 25% of the differential count and lymphocytes less than 15%. Increased neutrophils indicate a septic condition, whereas an elevated cell count with a predominance of lymphocytes suggests a nonseptic inflammation. In both normal and abnormal specimens, cells may appear more vacuolated than they do on a blood smear.³ Besides increased numbers of these usually

214 CHAPTER I2 • Synovial Fluid

normal cells, other cell abnormalities include the presence of eosinophils, LE cells, *Reiter cells* (vacuolated macrophages with ingested neutrophils), and RA cells or *ragocytes* (neutrophils with small, dark, cytoplasmic granules that consist of precipitated rheumatoid factor). Lipid droplets may be present following crush injuries, and hemosiderin granules are seen in cases of *pigmented villonodular synovitis*. The most frequently encountered cells and inclusions seen in synovial fluid are summarized in Table 12–4.

Crystal Identification

Microscopic examination of synovial fluid for the presence of crystals is an important diagnostic test in the evaluation of arthritis. Crystal formation in a joint frequently results in an acute, painful inflammation. It can also become a chronic condition. Causes of crystal formation include metabolic disorders and decreased renal excretion that produce elevated blood levels of crystallizing chemicals, degeneration of cartilage and bone, and injection of medications, such as corticosteroids into a joint.

Types of Crystals

The primary crystals seen in synovial fluid are monosodium urate (uric acid) (MSU) found in cases of gout and calcium pyrophosphate (CPPD) seen with pseudogout. Increased serum uric acid resulting from impaired metabolism of purines; increased consumption of high-purine-content foods, alcohol, and fructose; chemotherapy treatment of leukemias; and decreased renal excretion of uric acid are the most frequent causes of gout.⁷ Pseudogout is most often associated with degenerative arthritis, producing cartilage calcification and endocrine disorders that produce elevated serum calcium levels.

Additional crystals that may be present include hydroxyapatite (basic calcium phosphate) associated with calcified cartilage degeneration, cholesterol crystals associated with chronic inflammation, corticosteroids following injections, and calcium oxalate crystals in renal dialysis patients. Patient history must always be considered. Characteristics and significance of the commonly encountered crystals are presented in Table 12–5. Artifacts present may include talc and starch from gloves, precipitated anticoagulants, dust, and scratches on

Table 12-4 Cells and Inclusions Seen in Synovial Fluid			
Cell/Inclusion	Description	Significance	
Neutrophil	Polymorphonuclear leukocyte	Bacterial sepsis Crystal-induced inflammation	
Lymphocyte	Mononuclear leukocyte	Nonseptic inflammation	
Macrophage (monocyte)	Large mononuclear leukocyte, may be vacuolated	Normal Viral infections	
Synovial lining cell	Similar to macrophage, but may be multinucleated, resembling a <i>mesothelial cell</i>	Normal	
LE cell	Neutrophil containing characteristic ingested: "round body"	Lupus erythematosus	
Reiter cell	Vacuolated macrophage with ingested neutrophils	Reiter syndrome Nonspecific inflammation	
RA cell (ragocyte)	Neutrophil with dark cytoplasmic granules containing immune complexes	Rheumatoid arthritis Immunologic inflammation	
Cartilage cells	Large, multinucleated cells	Osteoarthritis	
Rice bodies	Macroscopically resemble polished rice Microscopically show collagen and fibrin	Tuberculosis, septic and rheumatoid arthritis	
Fat droplets	Refractile intracellular and extracellular globules Stain with Sudan dyes	Traumatic injury Chronic inflammation	
Hemosiderin	Inclusions within clusters of synovial cells	Pigmented villonodular synovitis	

Table 12-5 Characteristics of Synovial Fluid Crystals				
Crystal	Shape		Compensated Polarized Light	Significance
Monosodium urate	Needles		Negative birefringence	Gout
Calcium pyrophosphate	Rhombic square, rods		Positive birefringence	Pseudogout
Cholesterol	Notched, rhombic plates		Negative birefringence	Extracellular
Corticosteroid	Flat, variable-shaped plates		Positive and negative birefringence	Injections
Calcium oxalate	Envelopes		Negative birefringence	Renal dialysis
Apatite (Ca phosphate)	Small particles Require electron microscopy		No birefringence	Osteoarthritis

slides and coverslips. Slides and coverslips should be examined and cleaned prior to use.

Slide Preparation

Ideally, crystal examination should be performed soon after fluid collection to ensure that crystals are not affected by changes in temperature and pH. Both MSU and CPPD crystals are reported as being located extracellularly and intracellularly (within neutrophils); therefore, fluid must be examined prior to WBC disintegration.

Fluid is examined as an unstained wet preparation. One drop of fluid is placed on a precleaned glass slide and coverslipped. The slide can be initially examined under low and high power using a regular light microscope (Fig. 12-2). Crystals may be observed in Wright stained smears (Fig. 12-3), however this should not replace the wet prep examination and the use of polarized and *compensated polarized light* for identification.⁶

MSU crystals are routinely seen as needle-shaped crystals. They may be extracellular or located within the cytoplasm of neutrophils. They are frequently seen sticking through the cytoplasm of the cell.

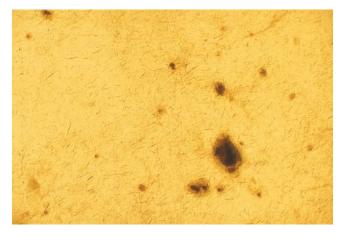


Figure 12–2 Unstained wet prep of MSU crystals (×400). Notice the characteristic yellow-brown color of urate crystals.

CPPD crystals usually appear rhombic-shaped or square but may appear as short rods. They are usually located within vacuoles of the neutrophils as shown in Figure 12-3. MSU crystals lyse phagosome membranes and therefore do not

216 CHAPTER 12 • Synovial Fluid

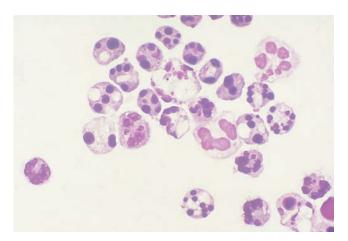


Figure 12–3 Wright stained neutrophils containing CPPD crystals (×1000).

appear in vacuoles.⁶ To avoid misidentification of CPPD crystals, the classic rhomboid shape should be observed and confirmed with compensated polarized microscopy.

Crystal Polarization

Once the presence of the crystals has been determined using direct polarization, positive identification is made using compensated polarized light. A control slide for the polarization properties of MSU can be prepared using betamethasone acetate corticosteroid.

Both MSU and CPPD crystals have the ability to polarize light, as discussed in Chapter 6; however, MSU is more highly birefringent and appears brighter against the dark background (Figs. 12-4 and 12-5).

When compensated polarized light is used, a red compensator is placed in the microscope between the crystal and the analyzer. The compensator separates the light ray into slow-moving and fast-moving vibrations and produces a red background (Fig. 12-6).

Owing to differences in the linear structure of the molecules in MSU and CPPD crystals, the color produced by each

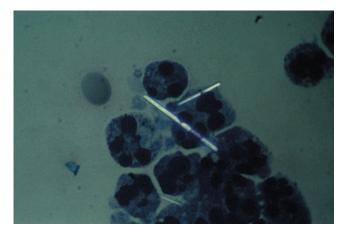


Figure 12–4 Strongly birefringent MSU crystals under polarized light (×500).

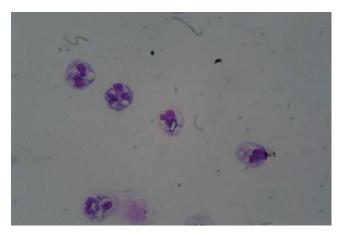


Figure 12–5 Weakly birefringent CPPD crystals under polarized light (×1000).

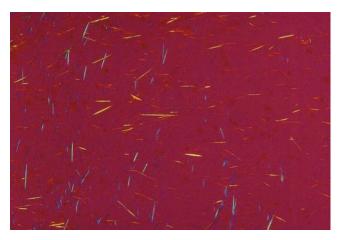


Figure 12–6 Extracellular MSU crystals under compensated polarized light: Notice the change in color with crystal alignment $(\times 100)$.

crystal when it is aligned with the slow vibration can be used to identify the crystal. The molecules in MSU crystals run parallel to the long axis of the crystal and, when aligned with the slow vibration, the velocity of the slow light passing through the crystal is not impeded as much as the fast light, which runs against the grain and produces a yellow color. This is considered negative birefringence (subtraction of velocity from the fast ray). In contrast, the molecules in CPPD crystals run perpendicular to the long axis of the crystal; when aligned with the slow axis of the compensator, the velocity of the fast light passing through the crystal is much quicker, producing a blue color and positive birefringence. 8 When the crystals are aligned perpendicular to the slow vibration, the color is reversed as shown in Figure 12-6. Care must be taken to ensure crystals being analyzed are aligned in accordance with the compensator axis. Notice how the colors of the MSU crystals in Figure 12-6 vary with the alignment. Figures 12-7 and 12-8 illustrate the characteristics of MSU and CPPD crystals under compensated polarized light.

Crystal shapes and patterns of birefringence that vary from the standard MSU and CPPD patterns may indicate the

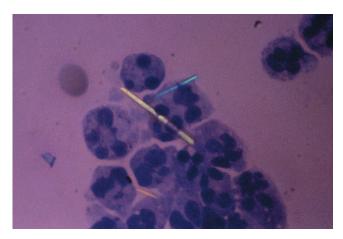


Figure 12–7 MSU crystals under compensated polarized light; the yellow crystal is aligned with the slow vibration $(\times 500)$.

presence of one of the less commonly encountered crystals and that further investigation is required (Fig. 12-9). Cholesterol, oxalate, and corticosteriod crystals exhibit birefringence, as do many contaminants. Apatite crystals are not birefringent.⁶

■ ■ ● Chemistry Tests

Because synovial fluid is chemically an ultrafiltrate of plasma, chemistry test values are approximately the same as serum values. Therefore, few chemistry tests are considered clinically important. The most frequently requested test is the glucose determination, as markedly decreased values are indicative of inflammatory (group 2) or septic (group 3) disorders. Because normal synovial fluid glucose values are based on the blood glucose level, simultaneous blood and synovial fluid samples should be obtained, preferably after the patient has fasted for 8 hours to allow equilibration between the two fluids. Under these conditions, normal synovial fluid glucose should not be more than 10 mg/dL lower than the

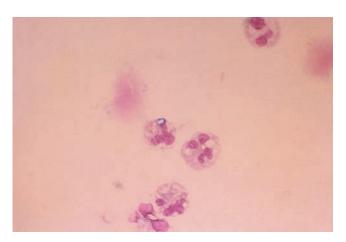


Figure 12–8 CPPD crystals under compensated polarized light, the blue crystal is aligned with the slow vibration $(\times 1000)$.

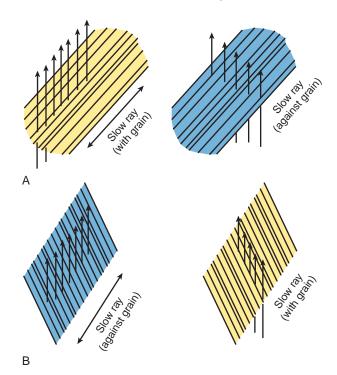


Figure 12–9 Diagram of negative and positive birefringence in MSU and CPPD crystals. (A) MSU crystal with grain running parallel to the long axis. The slow ray passes with the grain producing negative (yellow) birefringence. (B) CPPD crystal with grain running perpendicular to the long axis. The slow ray passes against the grain and is retarded producing positive (blue) birefringence.

blood value. To prevent falsely decreased values caused by glycolysis, specimens should be analyzed within 1 hour or preserved with sodium fluoride.

Other chemistry tests that may be requested are the total protein and uric acid determinations. Because the large protein molecules are not filtered through the synovial membranes, normal synovial fluid contains less than 3 g/dL of protein (approximately one third of the serum value). Increased levels are found in inflammatory and hemorrhagic disorders; however, measurement of synovial fluid protein does not contribute greatly to the classification of these disorders. When requested, the analysis is performed using the same methods used for serum protein determinations. The elevation of serum uric acid in cases of gout is well known; therefore, demonstration of an elevated synovial fluid uric acid level may be used to confirm the diagnosis when the presence of crystals cannot be demonstrated in the fluid. Measurement of serum uric acid is often performed as a first evaluaton of suspected cases of gout. Fluid analysis for crystals is frequently still required.

■■● Microbiologic Tests

An infection may occur as a secondary complication of inflammation caused by trauma or through dissemination of a systemic infection; therefore, Gram stains and cultures are two

218 CHAPTER I2 • Synovial Fluid

of the most important tests performed on synovial fluid. Both tests must be performed on all specimens, as organisms are often missed on Gram stain. Bacterial infections are most frequently seen; however, fungal, tubercular, and viral infections also can occur. When they are suspected, special culturing procedures should be used. Patient history and other symptoms can aid in requests for additional testing. Routine bacterial cultures should include an enrichment medium, such as chocolate agar, because in addition to *Staphylococcus* and *Streptococcus*, the common organisms that infect synovial fluid are the fastidious *Haemophilus* species and *N. gonorrhoeae*.

■■● Serologic Tests

Because of the association of the immune system to the inflammation process, serologic testing plays an important role in the diagnosis of joint disorders. However, the majority of the tests are performed on serum, with actual analysis of the synovial fluid serving as a confirmatory measure in cases that are difficult to diagnose. The autoimmune diseases rheumatoid arthritis and lupus erythematosus cause very serious inflammation of the joints and are diagnosed in the serology laboratory by demonstrating the presence of their particular autoantibodies in the patient's serum. These same antibodies can also be demonstrated in the synovial fluid, if necessary. Arthritis is a frequent complication of Lyme disease. Therefore, demonstration of antibodies to the causative agent Borrelia burgdorferi in the patient's serum can confirm the cause of the arthritis. The extent of inflammation can be determined through measurement of the concentration of acute phase reactants such as fibrinogen and C-reactive protein.

References

- 1. Shmerling, RH: Synovial fluid analysis. A critical reappraisal. Rheum Dis Clin North Am 20(2):503-512, 1994.
- Smith, GP, and Kjeldsberg, CR: Cerebrospinal, synovial, and serous body fluids. In Henry, JB (ed): Clinical Diagnosis and Management by Laboratory Methods. WB Saunders, Philadelphia, 2001.
- 3. Kjeldsberg, CR, and Knight, JA: Body Fluids: Laboratory Examination of Amniotic, Cerebrospinal, Seminal, Serous and Synovial Fluids: A Textbook Atlas. ASCP, Chicago, 1993.
- 4. Brown, W, et al: Validation of body fluid analysis on the Coulter LH 750. Lab Hem 9(3):155-159, 2004.
- 5. Kresie, L, et al: Evaluation of the application of body fluids on the sysmex XE 2100 series automated hematology analyzer. Lab Hem 11(1):24-30, 2005.
- 6. Schumacher, HD, Clayburne, G, and Chen, L: Synovial fluid aspiration and analysis in evaluation of gout and other crystal-induced diseases. Arthritis Foundation: Bulletin on the Rheumatic Diseases 53(3), 2004.
- 7. Harris, MD, Siegel, LB, and Alloway, JA: Gout and hyperuricemia. Amer Fam Physician 59(4):925-934, 1999.
- 8. Cornbleet, PJ: Synovial fluid crystal analysis. Lab Med 28(12):774-779, 1997.

QUESTIONS

- **1.** The functions of synovial fluid include all of the following *except*:
 - A. Lubrication for the joints
 - B. Removal of cartilage debris
 - C. Cushioning joints during jogging
 - D. Providing nutrients for cartilage
- **2.** The primary function of synoviocytes is to:
 - A. Provide nutrients for the joints
 - B. Secrete hyaluronic acid
 - C. Regulate glucose filtration
 - D. Prevent crystal formation
- **3.** Which of the following is not a frequently performed test on synovial fluid?
 - A. Uric acid
 - B. WBC count
 - C. Crystal examination
 - D. Gram stain
- **4.** The procedure for collection of synovial fluid is called:
 - A. Synovialcentesis
 - B. Arthrocentesis
 - C. Joint puncture
 - D. Arteriocentesis
- **5.** Match the following disorders with their appropriate group:
 - A. Noninflammatory
 - B. Inflammatory
 - C. Septic
 - D. Hemorrhagic

Gou

- ____N. gonorrhoeae infection
- Lupus erythematosus
- ____Osteoarthritis
- Hemophilia
- ____Rheumatoid arthritis
- ___Heparin overdose
- 6. Normal synovial fluid resembles:
 - A. Egg white
 - B. Normal serum
 - C. Dilute urine
 - D. Lipemic serum
- 7. Powdered anticoagulants should not be used in tubes for synovial fluid testing because it interferes with:
 - A. Cell counts
 - B. Glucose tests
 - C. Crystal examination
 - D. Differentials

- **8.** Addition of a cloudy, yellow synovial fluid to acetic acid produces a/an:
 - A. Yellow-white precipitate
 - B. Easily dispersed clot
 - C. Solid clot
 - D. Opalescent appearance
- **9.** To determine if a fluid is synovial fluid, it should be mixed with:
 - A. Sodium hydroxide
 - B. Hypotonic saline
 - C. Hyaluronidase
 - D. Acetic acid
- 10. The highest WBC count can be expected to be seen with:
 - A. Noninflammatory arthritis
 - B. Inflammatory arthritis
 - C. Septic arthritis
 - D. Hemorrhagic arthritis
- **11**. When diluting a synovial fluid WBC count, all of the following are acceptable *except*:
 - A. Acetic acid
 - B. Isotonic saline
 - C. Hypotonic saline
 - D. Saline with saponin
- **12**. The lowest percentage of neutophils would be seen in:
 - A. Noninflammatory arthritis
 - B. Inflammatory arthritis
 - C. Septic arthritis
 - D. Hemorrhagic arthritis
- **13**. All of the following are abnormal when seen in synovial fluid *except*:
 - A. RA cells
 - B. Reiter cells
 - C. Synovial lining cells
 - D. Lipid droplets
- **14.** Synovial fluid crystals that occur as a result of purine metabolism or chemotherapy for leukemia are:
 - A. Monosodium urate
 - B. Cholesterol
 - C. Calcium pyrophosphate
 - D. Apatite
- **15**. Synovial fluid crystals associated with inflammation in dialysis patients are:
 - A. Calcium pyrophosphate
 - B. Calcium oxalate
 - C. Corticosteroid
 - D. Monosodium urate
- 16. Crystals associated with pseudogout are:
 - A. Monosodium urate
 - B. Calcium pyrophosphate
 - C. Apatite
 - D. Corticosteroid

- 17. Synovial fluid for crystal examination should be examined as a/an:
 - A. Wet preparation
 - B. Wright stain
 - C. Gram stain
 - D. Acid-fast stain
- 18. Crystals that have the ability to polarize light are:
 - A. Corticosteroid
 - B. Monosodium urate
 - C. Calcium oxalate
 - D. All of the above
- 19. In an examination of synovial fluid under compensated polarized light, rhombic-shaped crystals are observed. What color would these crystals be when aligned parallel to the slow vibration?
 - A. White
 - B. Yellow
 - C. Blue
 - D. Red
- **20.** If crystals shaped like needles are aligned perpendicular to the slow vibration of compensated polarized light, what color are they?
 - A. White
 - B. Yellow
 - C. Blue
 - D. Red
- **21.** Negative birefringence occurs under compensated polarized light when:
 - A. Slow light is impeded more than fast light
 - B. Slow light is less impeded than fast light
 - C. Fast light runs against the molecular grain of the crystal
 - D. Both B and C
- **22.** Synovial fluid cultures are often plated on chocolate agar to detect the presence of:
 - A. Neisseria gonorrhoeae
 - B. Staphylococcus agalactiae
 - C. Streptococcus viridans
 - D. Enterococcus faecalis
- **23.** The most frequently performed chemical test on synovial fluid is:
 - A. Total protein
 - B. Uric acid
 - C. Calcium
 - D. Glucose
- **24**. Serologic tests on patients' serum may be performed to detect antibodies causing arthritis for all of the following disorders *except*:
 - A. Pseudogout
 - B. Rheumatoid arthritis
 - C. Lupus erythematosus
 - D. Lyme arthritis

220 CHAPTER 12 • Synovial Fluid

Continued

- **25.** Serologic testing of synovial fluid for fibrinogen and C-reactive protein is performed to:
 - A. Determine clot formation
 - B. Determine the amount of inflammation
 - C. Detect osteoarthritis
 - D. Diagnose rheumatoid arthritis

Case Studies and Clinical Situations

- 1. A 50-year-old man presents in the emergency room with severe pain and swelling in the right knee. Arthrocentesis is performed and 20 mL of milky synovial fluid is collected. The physician orders a Gram stain, culture, and crystal examination of the fluid, as well as a serum uric acid. He requests that the synovial fluid be saved for possible additional tests.
 - a.Describe the tubes into which the fluid would be routinely placed.
 - b.If the patient's serum uric acid level is elevated, what type of crystals and disorder are probable?
 - c. Describe the appearance of these crystals under direct and compensated polarized light.
 - d. Why were the Gram stain and culture ordered?
- **2.** A medical technology student dilutes a synovial fluid prior to performing a WBC count. The fluid forms a clot.
 - a. Why did the clot form?
 - b. How can the student perform a correct dilution of the fluid?
 - c.After the correct dilution is made, the WBC count is $100,000/\mu L$. State two arthritis classifications that could be considered.
 - d.State two additional tests that could be run to determine the classification.

3. Fluid obtained from the knee of an obese 65-year-old woman being evaluated for a possible knee replacement has the following results:

APPEARANCE: Pale yellow and hazy

WBC COUNT: 500 cells/mL GRAM STAIN: Negative

GLUCOSE: 110 mg/dL (serum glucose: 115 mg/dL) a. What classification of joint disorder do these results suggest?

- b. Under electron microscopy, what crystals might be detected?
- c. How does the glucose result aid in the disorder classification?
- **4.** A synovial fluid delivered to the laboratory for a cell count is clotted.
 - a. What abnormal constituent is present in the fluid?
 - b. What type of tube should be sent to the laboratory for a cell count?
 - c. Could the original tube be used for a Gram stain and culture? Why or why not?











S CHAPTER

Serous Fluid

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

- 1 Describe the normal formation of serous fluid.
- **2** Describe four primary causes of serous effusions.
- 3 Differentiate between a transudate and an exudate, including etiology, appearance, and laboratory tests.
- **4** Differentiate between a hemothorax and a hemorrhagic exudate.
- 5 Differentiate between a chylous and a pseudochylous exudate.
- 6 State the significance of increased neutrophils, lymphocytes, eosinophils, and plasma cells in pleural fluid.
- 7 Describe the morphologic characteristics of mesothelial cells and malignant cells.

- **8** List three common chemistry tests performed on pleural fluid, and state their significance.
- **9** State the common etiologies of pericardial effusions
- **10** Discuss the diagnostic significance of peritoneal lavage.
- 11 Calculate a serum-ascites gradient, and state its significance.
- 12 Differentiate between ascitic effusions of hepatic and peritoneal origin.
- 13 State the clinical significance of the carcinoembryonic antigen and CA 125 tests.
- **14** List four chemical tests performed on ascitic fluid, and state their significance.

KEY TERMS

ascites
effusion
exudate
hydrostatic pressure
oncotic pressure

paracentesis pericardiocentesis pericarditis parietal membrane peritonitis serous fluid
thoracentesis
transudate
visceral membrane

The closed cavities of the body—namely, the pleural, pericardial, and peritoneal cavities—are each lined by two membranes referred to as the serous membranes. One membrane lines the cavity wall (*parietal membrane*), and the other covers the organs within the cavity (*visceral membrane*). The fluid between the membranes is called *serous fluid*, and it provides lubrication between the parietal and visceral membranes. Lubrication is necessary to prevent the friction between the two membranes that occurs as a result of movement of the enclosed organs. An example of this movement

is the expansion and contraction of the lungs. Normally, only a small amount of serous fluid is present, because production and reabsorption take place at a constant rate.

■■● Formation

Serous fluids are formed as ultrafiltrates of plasma, with no additional material contributed by the mesothelial cells that line the membranes. Production and reabsorption are subject to hydrostatic and colloidal (oncotic) pressures from the cap-

222 CHAPTER 13 • Serous Fluid

illaries that serve the cavities and the capillary permeability. Under normal conditions, colloidal pressure from serum proteins is the same in the capillaries on both sides of the membrane. Therefore, the hydrostatic pressure in the parietal and visceral capillaries causes fluid to enter between the membranes. The filtration of the plasma ultrafiltrate results in increased oncotic pressure in the capillaries that favors reabsorption of fluid back into the capillaries. This produces a continuous exchange of serous fluid and maintains the normal volume of fluid beween the membranes. The slightly different amount of positive pressure in the parietal and visceral capillaries creates a small excess of fluid that is reabsorbed by the lymphatic capillaries located in the membranes. In Figure 13-1, the normal formation and absorption of pleural fluid are demonstrated.

Disruption of the mechanisms of serous fluid formation and reabsorption causes an increase in fluid between the membranes. This is termed an *effusion*. Primary causes of effusions include increased hydrostatic pressure (congestive heart failure), decreased oncotic pressure (hypoproteinemia), increased capillary permeability (inflammation and infection), and lymphatic obstruction (tumors) (Table 13–1).

Specimen Collection and Handling

Fluids for laboratory examination are collected by needle aspiration from the respective cavities. These aspiration procedures are referred to as *thoracentesis* (pleural), *pericardiocentesis* (pericardial), and *paracentesis* (peritoneal). Abundant fluid (greater than 100 mL) is usually collected;

Table 13–1

Pathologic Causes of Effusions

- Increased capillary hydrostatic pressure Congestive heart failure Salt and fluid retention
- 2. Decreased oncotic pressure Nephrotic syndrome Hepatic cirrhosis Malnutrition Protein-losing enteropathy
- Increased capillary permeability Microbial infections Membrane inflammations Malignancy
- 4. Lymphatic obstruction
 Malignant tumors, lymphomas
 Infection and inflammation
 Thoracic duct injury

therefore, suitable specimens are available for each section of the laboratory.

An ethylenediaminetetraacetic acid (EDTA) tube is used for cell counts and the differential. Sterile heparinized evacuated tubes are used for microbiology and cytology. For better recovery of microorganisms and abnormal cells, concentration of large amounts of fluid is performed by centrifugation. Chemistry tests can be run on clotted specimens in plain tubes or on heparinized tubes. Specimens for pH must be maintained anaerobically in ice. Chemical tests performed

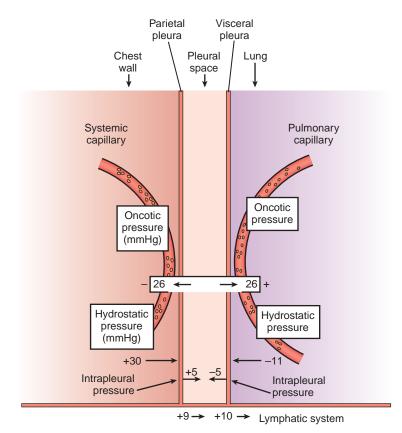


Figure 13–1 The normal formation and absorption of pleural fluid.

on serous fluids are frequently compared with plasma chemical concentrations because the fluids are essentially plasma ultrafiltrates. Therefore, blood specimens should be obtained at the time of collection.

Transudates and Exudates

A general classification of the cause of an effusion can be accomplished by separating the fluid into the category of transudate or exudate. Effusions that form because of a systemic disorder that disrupts the balance in the regulation of fluid filtration and reabsorption—such as the changes in hydrostatic pressure created by congestive heart failure or the hypoproteinemia associated with the nephrotic syndrome are called transudates. Exudates are produced by conditions that directly involve the membranes of the particular cavity, including infections and malignancies. Classification of a serous fluid as a transudate or exudate can provide a valuable initial diagnostic step and aid in the course of further laboratory testing, because testing of transudate fluids is usually not necessary.1 Traditionally, a variety of laboratory tests have been used to differentiate between transudates and exudates, including appearance, total protein, lactic dehydrogenase, cell counts, and spontaneous clotting. However, the most reliable differentiation is usually obtained by determining the fluidto-blood ratios for protein and lactic dehydrogenase.² Differential values for these parameters are shown in Table 13-2. Additional tests are available for specific fluids and will be discussed in the following sections.

■ ■ ● General Laboratory Procedures

Serous fluid examination—including classification as a transudate or exudate, appearance, cell count and differential, and chemistry, microbiology, and cytology procedures—is performed in the same manner on all serous fluids. However, the

significance of the test results and the need for specialized tests vary among fluids. Therefore, the interpretation of routine and special procedures will be discussed individually for each of the three serous fluids.

Tests that are usually performed on all serous fluids include evaluation of the appearance and differentiation between a transudate and an exudate. Effusions of exudative origin are then examined for the presence of microbiologic and cytologic abnormalities. Additional tests are ordered based on specific clinical symptoms. Red blood cell (RBC) and white blood cell (WBC) counts are not frequently performed on serous fluids because they provide little diagnostic information.3 In general, WBC counts greater than 1000/µL and RBC counts greater than 100,000/µL are indicative of an exudate. Serous fluid cell counts can be performed manually by using a Neubauer counting chamber and the methods discussed in Chapter 10 or by electronic cell counters (see Appendix A). Inclusion of tissue cells and debris in the count must be considered when electronic counters are used, and care must be taken to prevent the blocking of tubing with debris.

Differential cell counts are routinely performed on serous fluids, preferably on Wright's-stained, cytocentrifuged specimens or on slides prepared from the sediment of centrifuged specimens. Smears must be examined not only for WBCs, but also for normal and malignant tissue cells. Any suspicious cells seen on the differential are referred to the cytology laboratory or the pathologist.

■■● Pleural Fluid

Pleural fluid is obtained from the pleural cavity, located between the parietal pleural membrane lining the chest wall and the visceral pleural membrane covering the lungs. Pleural effusions may be of either transudative or exudative origin. In addition to the tests routinely performed to differentiate between transudates and exudates, two additional

Table 13-2 Laboratory Differentiation of Transudates and Exudates		
	Transudate	Exudate
Appearance	Clear	Cloudy
Fluid:serum protein ratio	< 0.5	>0.5
Fluid:serum LD ratio	< 0.6	>0.6
WBC count	$<1000/\mu L$	>1000/μL
Spontaneous clotting	No	Possible
Pleural fluid cholesterol	<45-60 mg/dL	>45-60 mg/dL
Pleural fluid:serum cholesterol ratio	< 0.3	>0.3
Pleural fluid:bilirubin ratio	< 0.6	>0.6
Serum-ascites albumin gradient	>1.1	<1.1

224 CHAPTER 13 • Serous Fluid

Table 13–3 Correlation of Pleural Fluid Appearance and Disease ⁵	
Appearance	Disease
Clear, pale yellow	Normal
Turbid, white	Microbial infection (tuberculosis)
Bloody	Hemothorax Hemorrhagic effusion, pulmonary embolis, tuberculosis, malignancy
Milky	Chylous material from thoracic duct leakage Pseudochylous material from chronic inflammation
Brown	Rupture of amoebic liver abscess
Black	Aspergillous
Viscous	Malignant mesothelioma (increased hyaluronic acid)

procedures are helpful when analyzing pleural fluid. These are the pleural fluid cholesterol and fluid:serum cholesterol ratio and the pleural fluid:serum total bilirubin ratio. A pleural fluid cholesterol greater than 60 mg/dL or a pleural fluid: serum cholesterol ratio greater than 0.3 provides reliable information that the fluid is an exudate. A fluid:serum total bilirubin ratio of 0.6 or more also indicates the presence of an exudate.

Appearance

Considerable diagnostic information concerning the etiology of a pleural effusion can be learned from the appearance of the specimen (Table 13–3). Normal and transudate pleural fluids are clear and pale yellow. Turbidity is usually related to the presence of WBCs and indicates bacterial infection, tuberculosis, or an immunologic disorder such as rheumatoid arthritis. The presence of blood in the pleural fluid can signify a *hemothorax* (traumatic injury), membrane damage such as occurs in malignancy, or a traumatic aspiration. As seen

with other fluids, blood from a traumatic tap appears streaked and uneven.

To differentiate between a hemothorax and hemorrhagic exudate, a hematocrit can be run on the fluid. If the blood is from a hemothorax, the fluid hematocrit is more than 50% of the whole blood hematocrit, because the effusion is actually occurring from the inpouring of blood from the injury.⁵ A chronic membrane disease effusion contains both blood and increased pleural fluid, resulting in a much lower hematocrit.

The appearance of a milky pleural fluid may be due to the presence of *chylous material* from thoracic duct leakage or to *pseudochylous material* produced in chronic inflammatory conditions. Chylous material contains a high concentration of triglycerides, whereas pseudochylous material has a higher concentration of cholesterol. Therefore, Sudan III staining is strongly positive with chylous material. In contrast, pseudochylous effusions contain cholesterol crystals. Differentiation between chylous and pseudochylous effusions is summarized in Table 13–4.

Table 13-4 Differentiation Between Chylous and Pseudochylous Pleural Effusions		
	Chylous Effusion	Pseudochylous Effusion
Cause	Thoracic duct leakage	Chronic inflammation
Appearance	Milky/white	Milky/green tinge
Leukocytes	Predominantly lymphocytes	Mixed cells
Cholesterol crystals	Absent	Present
Triglycerides	>110 mg/dL	<50 mg/dL
Sudan III staining	Strongly positive	Negative/weakly positive

Hematology Tests

As mentioned previously, the differential cell count is the most diagnostically significant hematology test performed on serous fluids. Primary cells associated with pleural fluid include neutrophils, lymphocytes, eosinophils, mesothelial cells, plasma cells, and malignant cells (Table 13–5). These same cells are also found in pericardial and peritoneal fluids.

Similar to other body fluids, an increase in pleural fluid neutrophils is indicative of a bacterial infection, such as pneumonia. Neutrophils are also increased in effusions resulting from pancreatitis and pulmonary infarction.

Lymphocytes are normally noticeably present in both transudates and exudates in a variety of forms, including small, large, and reactive. More prominent nucleoli and cleaved nuclei may be present. Elevated lymphocyte counts are seen in effusions resulting from tuberculosis, viral infections, malignancy, and autoimmune disorders such as rheumatoid arthritis and systemic lupus erythematosus. LE cells may be seen (Fig. 13-2).

Increased eosinophil levels (greater than 10%) may be associated with trauma resulting in the presence of air or blood (pneumothorax and hemothorax) in the pleural cavity. They are also seen in allergic reactions and parasitic infections.

The membranes lining the serous cavities contain a single layer of mesothelial cells; therefore, it is not unusual to find these cells in the serous fluids. Mesothelial cells are pleomorphic; they resemble lymphocytes, plasma cells, and malignant cells, frequently making identification difficult. They often appear as single, small, or large round cells with abundant blue cytoplasm and round nuclei with uniform

Table 13–5	Significance of Cells Seen in Pleural Fluid
Cell	Significance
Neutrophils	Pneumonia Pancreatitis Pulmonary infarction
Lymphocytes	Tuberculosis Viral infection Autoimmune disorders Malignancy
Mesothelial cells	Normal and reactive forms have no clinical significance Decreased mesothelial cells are associated with tuberculosis
Plasma cells	Tuberculosis
Malignant cells	Primary adenocarcinoma and small- cell carcinoma Metastatic carcinoma

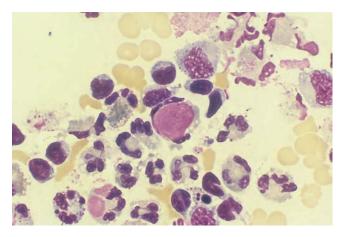


Figure 13–2 LE cell in pleural fluid. Notice the ingested "round body" (×1000).

dark purple cytoplasm and may be referred to as "normal" mesothelial cells (Figs. 13-3 and 13-4). In contrast, "reactive" mesothelial cells may appear in clusters; have varying amounts of cytoplasm, eccentric nuclei, and prominent nucleoli; and be multinucleated, and thus more closely resemble malignant cells (Figs. 13-5 and 13-6). An increase in mesothelial cells is not a diagnostically significant finding; however, they may be increased in pneumonia and malignancy. Of more significance is the noticeable lack of mesothelial cells associated with tuberculosis, which results from exudate covering the pleural membranes. Also associated with tuberculosis is an increase in the presence of pleural fluid plasma cells (Fig. 13-7).

A primary concern in the examination of all serous effusions is detecting the presence of malignant cells. Differentiation among mesothelial cells and other tissue cells and malignant cells is often difficult. Distinguishing characteristics of malignant cells may include nuclear and cytoplasmic irregularities, hyperchromatic nucleoli, cellular clumps with cytoplasmic molding (community borders), and abnormal nuclear-to-cytoplasmic ratios (Figs. 13-8 to 13-10). Malignant

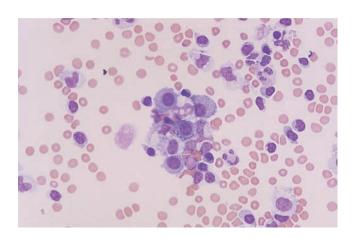


Figure 13–3 Normal pleural fluid mesothelial cells, lymphocytes, and monocytes (×250).

226 CHAPTER 13 • Serous Fluid

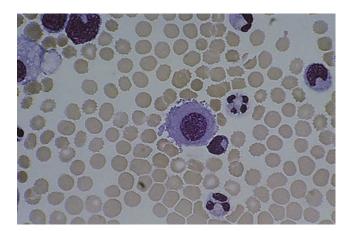


Figure 13-4 Normal mesothelial cell (×500).

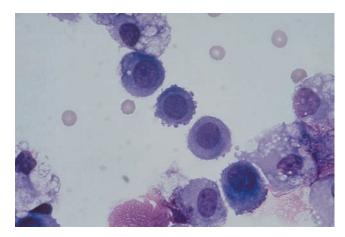


Figure 13–5 Reactive mesothelial cells showing eccentric nuclei and vacuolated cytoplasm (×500).

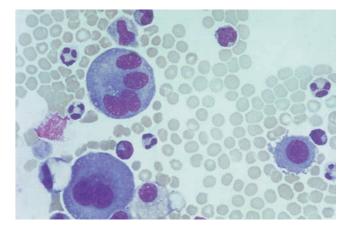


Figure 13–6 One normal and two reactive mesothelial cells with a multinucleated form (×500).

pleural effusions most frequently contain large, irregular adenocarcinoma cells, small or oatcell carcinoma cells resembling large lymphocytes, and clumps of metastatic breast carcinoma cells (Figs. 13-11 to 13-13). Special staining techniques and flow cytometry may be used for positive identification of

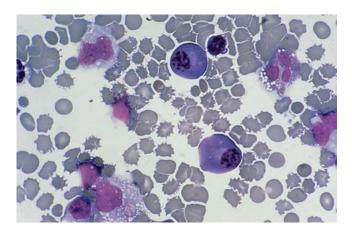


Figure 13–7 Pleural fluid plasma cells seen in a case of tuberculosis. Notice the absence of mesothelial cells (×1000).

tumor cells. Table 13–6 describes the primary characteristics of malignant serous fluid cells.

Chemistry Tests

In addition to the chemical tests performed to differentiate between a pleural transudate and exudate, the most common chemical tests performed on pleural fluid are glucose, pH, adenosine deaminase (ADA), and amylase. Triglyceride levels may also be measured to confirm the presence of a chylous effusion.

Decreased glucose levels are seen with tuberculosis, rheumatoid inflammation, and purulent infections. As an ultrafiltrate of plasma, pleural fluid glucose levels parallel plasma levels with values less than 60 mg/dL considered decreased. Fluid values should be compared with plasma values. Pleural fluid lactate levels are elevated in bacterial infections and can be considered in addition to the glucose level.

Pleural fluid pH lower than 7.0 may indicate the need for chest-tube drainage, in addition to administration of

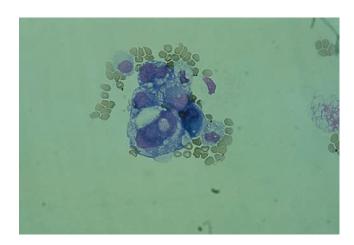


Figure 13–8 Pleural fluid adenocarcinoma showing cytoplasmic molding (×250).

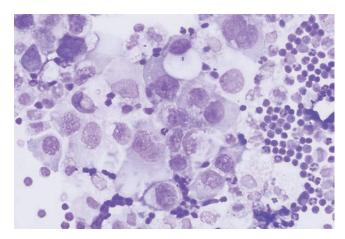


Figure 13–9 Pleural fluid adenocarcinoma showing nuclear and cytoplasmic molding, and vacuolated cytoplasm (×1000).

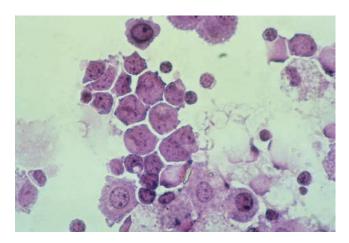


Figure 13–10 Enhancement of nuclear irregularities using a toluidine blue stain (\times 250).

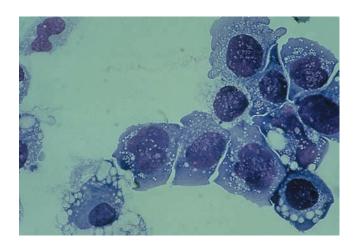


Figure 13–11 Poorly differentiated pleural fluid adenocarcinoma showing nuclear irregularities and cytoplasmic vacuoles (×500).

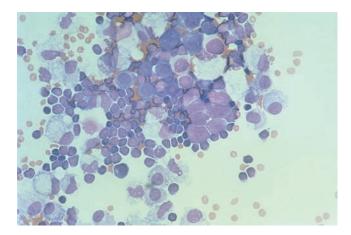


Figure 13–12 Pleural fluid small-cell carcinoma showing nuclear molding (×250).

antibiotics in cases of pneumonia. In cases of acidosis, the pleural fluid pH should be compared to the blood pH. Pleural fluid pH at least 0.30 degrees lower than the blood pH is considered significant.⁶ The finding of a pH as low as 6.0 indicates an esophageal rupture that is allowing the influx of gastric fluid.

ADA levels over 40 U/L are highly indicative of tuberculosis. They are also frequently elevated with malignancy.

As with serum, elevated amylase levels are associated with pancreatitis, and amylase is often elevated first in the pleural fluid. Pleural fluid amylase, including salivary amylase, may also be elevated in esophageal rupture and malignancy.

Microbiologic and Serologic Tests

Microorganisms primarily associated with pleural effusions include *Staphylococcus aureus*, Enterobacteriaceae, anaerobes, and *Mycobacterium tuberculosis*. Gram stains, cultures (both aerobic and anaerobic), acid-fast stains, and mycobacteria cultures are performed on pleural fluid when clinically indicated. Serologic testing of pleural fluid is used to differentiate

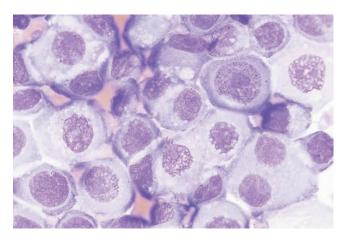


Figure 13–13 Metastatic breast carcinoma cells in pleural fluid. Notice the hyperchromatic nucleoli (×1000).

228 CHAPTER 13 • Serous Fluid

Table 13-6 Characteristics of Malignant Cells

- 1. Increased nucleus:cytoplasm (N:C) ratio. The higher the ratio the more poorly differentiated are the cells
- 2. Irregularly distributed nuclear chromatin
- 3. Variation in the size and shape of nuclei
- 4. Increased number and size of nucleoli
- 5. Hyperchromatic nucleoli
- 6. Giant cells and multinucleation
- 7. Nuclear molding
- 8. Cytoplasmic molding (community borders)
- 9. Vacuolated cytoplasm, mucin production
- 10. Cellular crowding, phagocytosis

effusions of immunologic origin from noninflammatory processes. Tests for antinuclear antibody (ANA) and rheumatoid factor (RF) are the most frequently performed.

Detection of the tumor markers carcinoembryonic antigen (CEA), CA 125 (metastatic uterine cancer), CA15.3 and CA 549 (breast cancer), and CYFRA 21-1 (lung cancer) provide valuable diagnostic information in effusions of malignant origin.⁷

Testing of pleural fluid and its significance is summarized in Figure 13-14.

Pericardial Fluid

Normally, only a small amount (10 to 50 mL) of fluid is found between the pericardial serous membranes. Pericardial effusions are primarily the result of changes in the permeability of the membranes due to infection (*pericarditis*), malignancy, and trauma-producing exudates. Metabolic disorders such as uremia, hypothyroidism, and autoimmune disorders are the primary causes of transudates (Table 13–7). The presence of an effusion is suspected when cardiac compression (tamponade) is noted during the physician's examination. See Table 13–8.

Appearance

Normal and transudate pericardial fluid appears clear and pale yellow. Effusions resulting from infection and malignancy are turbid, and malignant effusions are frequently blood streaked. Grossly bloody effusions are associated with accidental cardiac puncture and misuse of anticoagulant medications. Milky fluids representing chylous and pseudochylous effusions may also be present.

Laboratory Tests

Tests performed on pericardial fluid are primarily directed at determining if the fluid is a transudate or an exudate and include the fluid:serum protein and lactic dehydrogenase

PLEURAL FLUID TESTING ALGORITHM

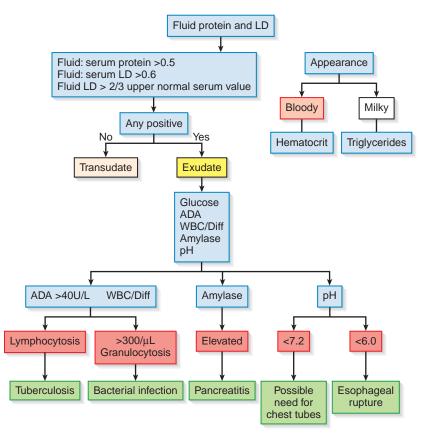


Figure 13-14 Algorithm of pleural fluid testing.

Table 13–7	Significance of Chemical Testing of Pleural Fluid
Test	Significance
Glucose	Decreased in rheumatoid inflammation Decreased in purulent infection
Lactate	Elevated in bacterial infection
Triglyceride	Elevated in chylous effusions
рН	Decreased in pneumonia not responding to antibiotics Markedly decreased with esophageal rupture
ADA	Elevated in tuberculosis and malignancy
Amylase	Elevated in pancreatitis, esophageal rupture, and malignancy

(LD) ratios. Like pleural fluid, WBC counts are of little clinical value, although a count of greater than 1000 WBCs/ μ L with a high percentage of neutrophils can be indicative of bacterial endocarditis.

Cytologic examination of pericardial exudates for the presence of malignant cells is an important part of the fluid analysis. Cells most frequently encountered are the result of

Table 13–8 Signi	ficance of Pericardial
THE RESERVE OF THE PARTY OF THE	Testing
Test	Significance
Appearance	
Clear, pale yellow	Normal, transudate
Blood-streaked	Infection, malignancy
Grossly bloody	Cardiac puncture, anticoagulant medications
Milky	Chylous and pseudochylous material
Differential	
Increased neutrophils	Bacterial endocarditis
Malignant cells	Metastatic carcinoma
Carcinoembryonic antigen	Metastatic carcinoma
Gram stain and culture	Bacterial endocarditis
Acid-fast stain	Tubercular effusion
Adenosine deaminase	Tubercular effusion

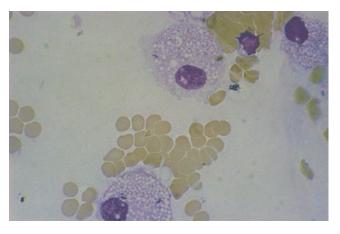


Figure 13–15 Malignant pericardial effusion showing giant mesothelioma cell with cytoplasmic molding and hyper-chromatic nucleoli (×1000).

metastatic lung or breast carcinoma and resemble those found in pleural fluid. Figure 13-15 represents a metastatic giant mesothelioma cell that is frequently seen in pleural fluid and is associated with asbestos contact. Pericardial fluid tumor marker levels correlate well with cytologic studies.⁷

Bacterial cultures and Gram stains are performed on concentrated fluids when endocarditis is suspected. Infections are frequently caused by previous respiratory infections including *Haemophilus*, *Streptococcus*, *Staphylococcus*, Adenovirus, and Coxsackievirus. Effusions of tubercular origin are increasing as a result of AIDS. Therefore, acid-fast stains and chemical tests for adenosine deaminase are often requested on pericardial effusions.

■■● Peritoneal Fluid

Accumulation of fluid between the peritoneal membranes is called *ascites*, and the fluid is commonly referred to as ascitic fluid rather than peritoneal fluid. In addition to the causes of transudative effusions discussed previously, hepatic disorders such as *cirrhosis* are frequent causes of ascitic transudates. Bacterial infections (*peritonitis*)—often as a result of intestinal perforation or a ruptured appendix—and malignancy are the most frequent causes of exudative fluids (Table 13–9).

Normal saline is sometimes introduced into the peritoneal cavity to act as a lavage for the detection of abdominal injuries that have not yet resulted in the accumulation of fluid. *Peritoneal lavage* is a sensitive test for the detection of intra-abdominal bleeding in blunt trauma cases, and results of the RBC count can be used along with radiographic procedures to aid in determining the need for surgery. RBC counts greater than $100,000/\mu L$ are indicative of blunt trauma injuries.

Transudates Versus Exudates

Differentiation between ascitic fluid transudates and exudates is more difficult than for pleural and pericardial effusions. The

230 CHAPTER 13 • Serous Fluid

	nificance of Peritoneal d Testing
Test	Significance
Appearance	
Clear, pale yellow	Normal
Turbid	Microbial infection
Green	Gallbladder, pancreatic disorders
Blood-streaked	Trauma, infection, or malignancy
Milky	Lymphatic trauma and blockage
Peritoneal lavage	>100,000 RBCs/µL indicates blunt trauma injury
WBC count	
<500 cells/μL	Normal
>500 cells/μL	Bacterial peritonitis, cirrhosis
Differential	Bacterial peritonitis
	Malignancy
Carcinoembryonic antigen	Malignancy of gastrointestinal origin
CA 125	Malignancy of ovarian origin
Glucose	Decreased in tubercular peritonitis, malignancy
Amylase	Increased in pancreatitis, gastrointestinal perforation
Alkaline phosphatase	Increased in gastrointestinal perforation
Blood urea nitrogen/ creatinine	Ruptured or punctured bladder
Gram stain and culture	Bacterial peritonitis
Acid-fast stain	Tubercular peritonitis
Adenosine deaminase	Tubercular peritonitis

serum-ascites albumin gradient (SAAG) is recommended over the fluid:serum total protein and LD ratios for the detection of transudates of hepatic origin⁸ Fluid and serum albumin levels are measured concurrently, and the fluid albumin level is then subtracted from the serum albumin level. A difference (gradient) of 1.1 or greater suggests a transudate effusion of hepatic origin, and lower gradients are associated with exudative effusions.

Example:

Serum albumin Serum albumin = 3.8 mg/dL = 3.8 mg/dL Fluid albumin Fluid albumin = 1.2 mg/dL = 3.0 mg/dL Gradient = 2.6 Gradient = 0.8 transudate effusion

Appearance

Like pleural and pericardial fluids, normal peritoneal fluid is clear and pale yellow. Exudates are turbid with bacterial or fungal infections. Green or dark brown color indicates the presence of bile, which can be confirmed using standard chemical tests for bilirubin. Blood-streaked fluid is seen following trauma and with tuberculosis, intestinal disorders, and malignancy. Chylous or pseudochylous material may be present with trauma or blockage of lymphatic vessels.

Laboratory Tests

Normal WBC counts are less than 350 cells/ μ L, and the count increases with bacterial peritonitis and cirrhosis. To distinguish between those two conditions, an absolute neutrophil count should be performed. An absolute neutrophil count greater than 250 cells/ μ L or greater than 50% of the total WBC count is indicative of infection. Lymphocytes are the predominant cell in tuberculosis.

Cellular Examination

Examination of ascitic exudates for the presence of malignant cells is important for the detection of tumors of primary and metastatic origin. Malignancies are most frequently of gastrointestinal, prostate, or ovarian origin. Cells present in ascitic fluid include leukocytes, abundant mesothelial cells, and macrophages, including lipophages (Fig. 13-16). Microorganisms including bacteria, yeast, and Toxoplasma gondii may also be present (Fig. 13-17). Malignant cells of ovarian, prostatic, and colonic origin, often containing mucin-filled vacuoles, are frequently seen (Figs. 13-18 to 13-21). Psammoma bodies containing concentric striations of collagen-like material can be seen in benign conditions and are also associated with ovarian and thyroid malignancies (Fig. 13-22). Measurement of the tumor markers CEA and CA 125 is a valuable procedure for identifying the primary source of tumors producing ascitic exudates. The presence of CA 125 antigen with a negative CEA suggests the source is from the ovaries, fallopian tubes, or endometrium.6

Chemical Testing

Chemical examination of ascitic fluid consists primarily of glucose, amylase, and alkaline phosphatase determinations. Glucose is decreased below serum levels in bacterial and

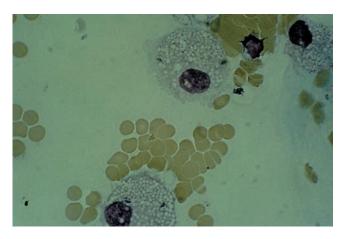


Figure 13–16 Lipophages (macrophages containing fat droplets) in peritoneal fluid (×500).

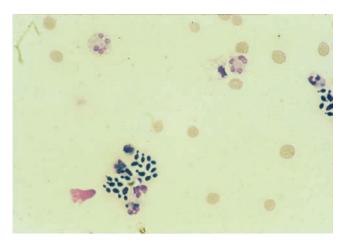


Figure 13–17 Budding yeast in peritoneal fluid (×400).

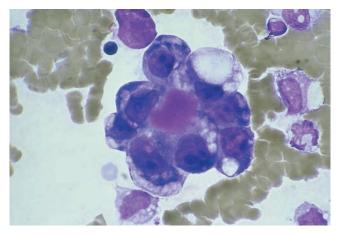


Figure 13–18 Ovarian carcinoma showing community borders, nuclear irregularity, and hyperchromatic nucleoli $(\times 500)$.

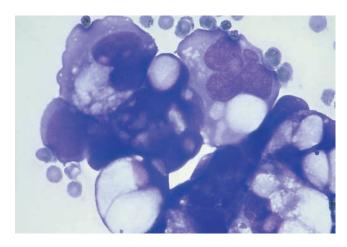


Figure 13–19 Ovarian carcinoma cells with large mucin-containing vacuoles (×500).

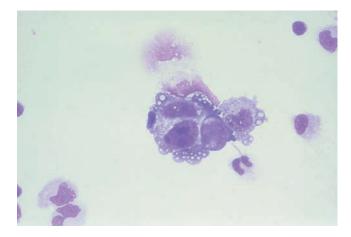


Figure 13–20 Adenocarcinoma of the prostate showing cytoplasmic vacuoles, community borders, and hyperchromatic nucleoli (×500).

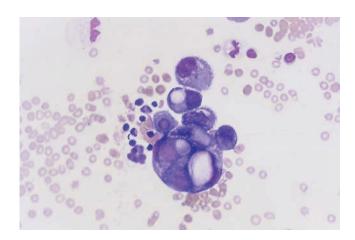


Figure 13–21 Colon carcinoma cells containing mucin vacuoles and nuclear irregularities.

232 CHAPTER 13 • Serous Fluid

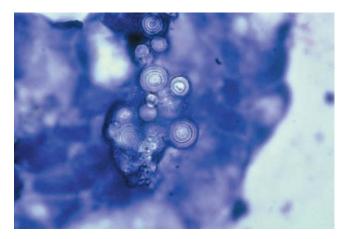


Figure 13–22 Psammoma bodies exhibiting concentric striations (×500).

tubercular peritonitis and malignancy. Amylase is determined on ascitic fluid to ascertain cases of pancreatitis, and it may be elevated in patients with gastrointestinal perforations. An elevated alkaline phosphatase level is also highly diagnostic of intestinal perforation.

Measurements of blood urea nitrogen and creatinine in the fluid are requested when a ruptured bladder or accidental puncture of the bladder during the paracentesis is of concern.

Microbiology Tests

Gram stains and bacterial cultures for both aerobes and anaerobes are performed when bacterial peritonitis is suspected. Inoculation of fluid into blood culture bottles at the bedside increases the recovery of anaerobic organisms. Acid-fast stains, adenosine deaminase, and cultures for tuberculosis may also be requested.

References

- 1. Jay, SJ: Pleural effusions: Definitive evaluation of the exudate. Postgrad Med 80(5):180-188, 1986.
- 2. Light, RW: Clinical practice: Pleural effusion. N Engl J Med 346:1971-1977, 2002.
- 3. Kjeldsberg, CR, and Knight, JA: Body Fluids: Laboratory Examination of Amniotic, Cerebrospinal, Seminal, Serous and Synovial Fluids. A Textbook Atlas. ASCP, Chicago, 1993.
- 4. Valdez, L, et al: Cholesterol: A useful parameter for distinguishing between pleural exudates and transudates. Chest 99(5): 1097-1102, 1991.
- 5. Porcel, JM, and Light, RW: Diagnostic approach to pleural fluid effusion in adults. Am Fam Physician 73:1211-1220, 2006.
- Colice, GL, et al: Medical and surgical treatment of parapneumonic effusions: An evidence-based guideline. Chest 118:1158-1171, 2000.
- 7. Porcel, JM, et al: Use of a panel of tumor markers (carcinoembryonic antigen, cancer antigen 125, carbohydrate antigen 15-3 and cytokeratin 19 fragments) in pleural fluid for differential diagnosis of benign and malignant effusions. Chest 126:1757-1763, 2004.
- 8. Runyan, BA, et al: The serum-ascites albumin gradient is superior to the exudate transudate concept in the differential diagnosis of ascites. Ann Intern Med 117:215-218, 1992.

QUESTIONS

- 1. The primary purpose of serous fluid is:
 - A. Removal of waste products
 - B. Lowering of capillary pressure
 - C. Lubrication of serous membranes
 - D. Nourishing serous membranes
- 2. The membrane that lines the wall of a cavity is the:
 - A. Visceral
 - B. Peritoneal
 - C. Pleural
 - D. Parietal
- During normal production of serous fluid, the slight excess of fluid is:
 - A. Absorbed by the lymphatic system
 - B. Absorbed through the visceral capillaries
 - C. Stored in the mesothelial cells
 - D. Metabolized by the mesothelial cells
- **4.** Production of serous fluid is controlled by:
 - A. Capillary oncotic pressure
 - B. Capillary hydrostatic pressure
 - C. Capillary permeability
 - D. All of the above
- 5. An increase in the amount of serous fluid is called a/an:
 - A. Exudate
 - B. Transudate
 - C. Effusion
 - D. Malignancy
- **6.** Pleural fluid is collected by:
 - A. Pleurocentesis
 - B. Paracentesis
 - C. Pericentesis
 - D. Thoracentesis
- 7. Place the appropriate letter in front of the following statements describing transudates and exudates.
 - A. Transudate
 - B. Exudate
 - ____Caused by increased capillary permeability
 - ____Caused by increased hydrostatic pressure
 - ____Caused by decreased oncotic pressure
 - ____Caused by congestive heart failure
 - ____Malignancy related
 - ____Tuberculosis related
 - ____Nephrotic syndrome related
 - ____Cloudy appearance
- **8.** Fluid-to-serum protein and lactic dehydrogenase ratios are performed on serous fluids:
 - A. When malignancy is suspected
 - B. To classify transudates and exudates
 - C. To determine the type of serous fluid
 - D. When a traumatic tap has occurred

- **9.** Which of the following requires the most additional testing?
 - A. Transudate
 - B. Exudate
- **10**. An additional test performed on pleural fluid to classify the fluid as a transudate or exudate is the:
 - A. WBC count
 - B. RBC count
 - C. Fluid-to-cholesterol ratio
 - D. Fluid-to-serum protein gradient
- 11. A milky-appearing pleural fluid is indicative of:
 - A. Thoracic duct leakage
 - B. Chronic inflammation
 - C. Microbial infection
 - D. Both A and B
- **12**. Which of the following best represents a hemothorax?
 - A. Blood HCT: 42 Fluid HCT: 15
 - B. Blood HCT: 42 Fluid HCT: 10
 - C. Blood HCT: 30 Fluid HCT: 10
 - D. Blood HCT: 30 Fluid HCT: 20
- **13**. All of the following are normal cells seen in pleural fluid *except*:
 - A. Mesothelial cells
 - B. Neutrophils
 - C. Lymphocytes
 - D. Mesothelioma cells
- **14.** A differential observation of pleural fluid associated with tuberculosis is:
 - A. Increased neutrophils
 - B. Decreased lymphocytes
 - C. Decreased mesothelial cells
 - D. Increased mesothelial cells
- **15**. All of the following are characteristics of malignant cells *except*:
 - A. Cytoplasmic molding
 - B. Absence of nucleoli
 - C. Mucin-containing vacuoles
 - D. Increased N:C ratio
- 16. A pleural fluid pH of 6.0 is indicative of:
 - A. Esophageal rupture
 - B. Mesothelioma
 - C. Malignancy
 - D. Rheumatoid effusion
- 17. A mesothelioma cell seen in pleural fluid indicates:
 - A. Bacterial endocarditis
 - B. Primary malignancy
 - C. Metastatic lung malignancy
 - D. Tuberculosis infection

- 18. Another name for a peritoneal effusion is:
 - A. Peritonitis
 - B. Lavage
 - C. Ascites
 - D. Cirrhosis
- 19. The test performed on peritoneal lavage fluid is:
 - A. WBC count
 - B. RBC count
 - C. Absolute neutrophil count
 - D. Amylase
- **20**. The recommended test for determining if peritoneal fluid is a transudate or an exudate is the:
 - A. Fluid-to-serum albumin ratio
 - B. Serum ascites albumin gradient
 - C. Fluid-to-serum lactic dehydrogenase ratio
 - D. Absolute neutrophil count
- **21**. Given the following results, classify this peritoneal fluid: serum albumin, 2.2 g/dL; serum protein, 6.0 g/dL; fluid albumin, 1.6 g/dL.
 - A. Transudate
 - B. Exudate
- **22.** Differentiation between bacterial peritonitis and cirrhosis is done by performing a/an:
 - A. WBC count
 - B. Differential
 - C. Absolute neutrophil count
 - D. Absolute lymphocyte count
- **23**. Detection of the CA 125 tumor marker in peritoneal fluid is indicative of:
 - A. Colon cancer
 - B. Ovarian cancer
 - C. Gastric malignancy
 - D. Prostate cancer
- **24.** Chemical tests primarily performed on peritoneal fluid include all of the following *except*:
 - A. Lactose dehydrogenase
 - B. Glucose
 - C. Alkaline phosphatase
 - D. Amylase
- 25. Cultures of peritoneal fluid are incubated:
 - A. Aerobically
 - B. Anaerobically
 - C. At 37°C and 42°C
 - D. Both A and B

Case Studies and Clinical Situations

- 1. Fluid from a patient with congestive heart failure is collected by thoracentesis and sent to the laboratory for testing. It appears clear and pale yellow and has a WBC count of 450/mL, fluid:serum protein ratio of 0.35, and fluid:serum LD ratio of 0.46.
 - a. What type of fluid was collected?
 - b. Based on the laboratory results, would this fluid be considered a transudate or an exudate? Why?
 - c. List two other tests that could be performed to aid in classifying this fluid.
- 2. A cloudy pleural fluid has a glucose level of 30 mg/dL (serum glucose level is 100 mg/dL) and a pH of 6.8.
 - a. What condition do these results indicate?
 - b. What additional treatment might the patient receive based on these results?
- **3.** The following results were obtained on a peritoneal fluid: serum albumin, 2.8 g/dL; fluid albumin, 1.2 g/dL. a. Calculate the SAAG.
 - b. Is this a transudate or an exudate? Why?
 - c. What is the most probable cause of the effusion?

- 4. Paracentesis is performed on a patient with ascites. The fluid appears turbid and has an elevated WBC count. Additional tests ordered include an absolute granulocyte count, amylase, creatinine, CEA, and CA 125.
 - a. What is the purpose for the absolute granulocyte count? If it is less than 250 cells/mL, what condition is indicated?
 - b. If the amylase level is elevated, what is its significance? State an additional test that might be ordered.
 - c. Explain the significance of an elevated creatinine level.
 - d. What is the purpose of the CEA and CA 125 tests?
- 5. Describe a situation in which paracentesis might be performed on a patient who does not have ascites. If the RBC count is 300,000/mL, what does this indicate?
- **6.** Microscopic examination of an ascitic fluid shows many cells with nuclear and cytoplasmic irregularities containing Psammoma bodies. The CEA test result is normal. What additional test would be helpful?











CHAPTER CHAPTER

Amniotic Fluid

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

- 1 State the functions of amniotic fluid.
- **2** Describe the formation and composition of amniotic fluid.
- **3** State indications for performing an amniocentesis.
- 4 Describe the specimen-handling and processing procedures for testing amniotic fluid for bilirubin, fetal lung maturity (FLM), and cytogenetic analysis.
- **5** Discuss the principle of the spectrophotometric analysis for evaluation of hemolytic disease of the newborn.
- 6 Interpret a Liley graph.

- 7 Describe the analysis of amniotic fluid for the detection of neural tube disorders.
- **8** Explain the physiologic significance of the lecithin-sphingomyelin (L/S) ratio.
- **9** State the relationship of phosphatidyl glycerol to FLM.
- 10 Discuss the principle of and sources of error for the L/S ratio, Amniostat-FLM, Foam Stability Index, and microviscosity tests for FLM.
- 11 Describe the relationship of lamellar bodies to FLM and the laboratory tests performed.

KEY TERMS

amniocentesis cytogenetic analysis fetal lung maturity hemolytic disease of the newborn lamellar body lecithin-sphingomyelin ratio surfactants

Although the testing of amniotic fluid is frequently associated with *cytogenetic analysis*, the clinical laboratory also performs several significant tests on amniotic fluid. Because amniotic fluid is a product of fetal metabolism, the constituents that are present in the fluid provide information about the metabolic processes taking place during—as well as the progress of—fetal maturation. When conditions that adversely affect the fetus arise, the danger to the fetus must be measured against the ability of the fetus to survive an early delivery. The tests covered in this chapter are used to determine the extent of fetal distress and fetal maturity (Table 14–1).

Physiology

Function

Amniotic fluid is present in the *amnion*, a membranous sac that surrounds the fetus (Fig. 14-1). The primary functions of the fluid are to provide a protective cushion for the fetus, allow fetal movement, stablize the temperature to protect the fetus from extreme temperature changes, and to permit proper lung development. Exchanges of water and chemicals also take place between the fluid, the fetus, and the maternal circulation.

Table 14–1 Tests for Fetal Well-Being and Maturity		
Normal Values		
Test	at Term ⁷	Significance
Bilirubin scan	Δ A450 >.025	Hemolytic disease of the newborn
Alpha-fetoprotein	<2.0 MoM	Neural tube disorders
Lecithin-sphingomyelin ratio	≥2.0	Fetal lung maturity
Amniostat-fetal lung maturity	Positive	Fetal lung maturity/phosphatidyl glycerol
Foam Stability Index	≥47	Fetal lung maturity
Microviscosity (FLM-TDx)	≥55 mg/g	Fetal lung maturity
Optical density 650 nm	≥0.150	Fetal lung maturity
Lamellar body count	≥32,000/mL	Fetal lung maturity

Volume

Amniotic fluid volume is regulated by a balance between the production of fetal urine and lung fluid and the absorption from fetal swallowing and intramembranous flow. Intramembranous flow is the absorption of amniotic fluid water and solutes into the fetal vascular system.1 The amount of amniotic fluid increases throughout pregnancy, reaching a peak of approximately 1 L during the third trimester, and then gradually decreases prior to delivery. During the first trimester, the approximately 35 mL of amniotic fluid is derived primarily from the maternal circulation. During the latter third to half of pregnancy, the fetus secretes a volume of lung liquid necessary to expand the lungs with growth. During each episode of fetal breathing movement, secreted lung liquid enters the amniotic fluid, as evidenced by lung surfactants that serve as an index of fetal lung maturity.1 After the first trimester, fetal urine is the major contributor to the amniotic fluid volume. At the time that fetal urine production occurs, fetal swallowing of the amniotic fluid begins and regulates the increase in fluid from the fetal urine.

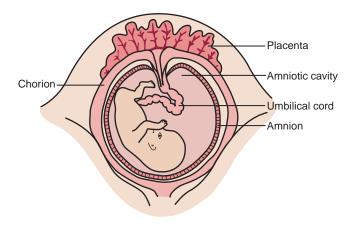


Figure 14-1 Fetus in amniotic sac.

Failure of the fetus to begin swallowing results in excessive accumulation of amniotic fluid (*polyhydramnios*) and is an indication of fetal distress, often associated with neural tube disorders. Polyhydramnios may be secondarily associated with fetal structural anomalies, cardiac arrhythmias, congenital infections, or chromosomal abnormalities. Increased fetal swallowing, urinary tract deformities, and membrane leakage are possible causes of decreased amniotic fluid (*oligohydramnios*). Oligohydramnios may be associated with umbilical cord compression, resulting in decelerated heart rate and fetal death. 1

Chemical Composition

The placenta is the ultimate source of amniotic fluid water and solutes. Amniotic fluid has a composition similar to that of the maternal plasma and contains a small amount of sloughed fetal cells from the skin, digestive system, and urinary tract. These cells provide the basis for cytogenetic analysis. The fluid also contains biochemical substances that are produced by the fetus, such as bilirubin, lipids, enzymes, electrolytes, nitrogenous compounds, and proteins that can be tested to determine the health or maturity of the fetus.

A portion of the fluid arises from the fetal respiratory tract, fetal urine, the amniotic membrane, and the umbilical cord. As would be expected, the chemical composition of the amniotic fluid changes when fetal urine production begins. The concentrations of creatinine, urea, and uric acid increase, whereas glucose and protein concentrations decrease. Concentrations of electrolytes, enzymes, hormones, and metabolic end products also vary but are of little clinical significance. Measurement of amniotic fluid creatinine has been used to determine fetal age. Prior to 36 weeks' gestation, the amniotic fluid creatinine level ranges between 1.5 and 2.0 mg/dL. It then rises above 2.0 mg/dL, thereby providing a means of determining fetal age as greater than 36 weeks.²

Differentiating Maternal Urine From Amniotic Fluid

Differentiation between amniotic fluid and maternal urine may be necessary to determine possible premature membrane rupture or accidental puncture of the maternal bladder during specimen collection. Chemical analysis of creatinine, urea, glucose, and protein aids in the differentiation. Levels of creatinine and urea are much lower in amniotic fluid than in urine. Creatinine does not exceed 3.5 mg/dL and urea does not exceed 30 mg/dL in amniotic fluid, whereas values as high as 10 mg/dL for creatinine and 300 mg/dL for urea may be found in urine.³ Measurement of glucose and protein by a reagent strip is a less reliable indicator, because glucose and protein are not uncommon urine constituents during pregnancy. However, under normal circumstances, the presence of glucose, protein, or both is associated more closely with amniotic fluid.

The fern test also can differentiate amniotic fluid from urine. In the fern test, a vaginal fluid specimen is spread on a glass slide and allowed to completely air dry at room temperature, then is observed microscopically. The presence of "fern-like" crystals is a positive screen for amniotic fluid.⁴

Specimen Collection

Indications for Amniocentesis

Amniocentesis is recommended when screening blood tests such as the maternal serum alpha fetal protein test, the triple screening test (tests for maternal alpha fetal protein [AFP], human chorionic gonadotropin [hCG], and unconjugated estriol [UE3]), or the quadruple screening test (AFP, hCG, UE3, and inhibin A) yield results that are abnormal. Fetal body measurements taken with ultrasonography accurately estimate the gestational age of the fetus and provide an assessment of the size and growth of the fetus throughout pregnancy to diagnose and manage intrauterine growth retardation. Finding an abnormality on the ultrasound could indicate potential fetal development problems and indicate the need for an amniocentesis and laboratory measurements of fetal lung maturity.

Fetal epithelial cells in amniotic fluid indicate the genetic material of the fetus and the biochemical substances that the fetus has produced. These cells can be separated from the fluid, cultured, and examined for chromosome abnormalities by karyotyping, fluorescence in situ hybridization (FISH), fluorescent mapping spectral karyotyping (SKY), and DNA testing. Biochemical substances produced by the fetus can be analyzed by fluorescence polarization and thin-layer chromatography to evaluate the health of the fetus.

Indications for Performing Amniocentesis

Amniocentesis may be indicated at 15 to 18 weeks of gestation for the following conditions to determine early treatment or intervention:

- Mother's age of 35 or more at delivery
- Family history of chromosome abnormalities, such as trisomy 21 (Down syndrome)

- Parents carry an abnormal chromosome rearrangement
- Earlier pregnancy or child with birth defect
- Parent is a carrier of a metabolic disorder
- History of genetic diseases such a sickle cell disease, Tay-Sachs disease, hemophilia, muscular dystrophy, sickle cell anemia, Huntington chorea, and cystic fibrosis
- Elevated maternal serum alpha fetoprotein
- Abnormal triple marker screening test
- Previous child with a neural tube disorder such as spina bifida, or ventral wall defects (gastroschisis)
- Three or more miscarriages

Evaluation of amniocentesis is indicated later in the pregnancy (20 to 42 weeks) to evaluate:

- · Fetal lung maturity
- · Fetal distress
- Hemolytic disease of the newborn caused by Rh blood type incompatibility
- Infection

Collection

Amniotic fluid is obtained by needle aspiration into the amniotic sac, a procedure called *amniocentesis*. The procedure most frequently performed is a transabdominal amniocentesis. Using continuous ultrasound for guidance, the physician locates the fetus and placenta to safely perform the procedure. A thin, hollow needle is inserted through the mother's abdomen into the mother's uterus and into the amniotic sac to aspirate the amniotic fluid. Vaginal amniocentesis may also be performed; however, this method carries a greater risk of infection. In general, amniocentesis is a safe procedure, particularly when performed after the 14th week of gestation. Fluid for chromosome analysis is usually collected at approximately 16 weeks' gestation, whereas tests for fetal distress and maturity are performed later in the third trimester.

A maximum of 30 mL of amniotic fluid is collected in sterile syringes. The first 2 or 3 mL collected can be contaminated by maternal blood, tissue fluid, and cells and are discarded. Fluid for bilirubin analysis in cases of *hemolytic disease of the newborn* (HDN) must be protected from light at all times. This can be accomplished by placing the specimens in amber-colored tubes or by use of a black plastic cover for the specimen container.

Specimen Handling and Processing

Handling and processing of amniotic fluid vary with the tests requested. However, in all circumstances, special handling procedures should be performed immediately and the specimen delivered promptly to the laboratory. Fluid for *fetal lung*

238 CHAPTER 14 • Amniotic Fluid

maturity (FLM) tests should be placed in ice for delivery to the laboratory and refrigerated up to 72 hours prior to testing or kept frozen and tested within 72 hours. Frozen specimens should be thoroughly mixed, by vortexing, after thawing. Repeated freeze-thawing is not recommended.⁵ Specimens for cytogenetic studies are maintained at room temperature or body temperature (37°C incubation) prior to analysis to prolong the life of the cells needed for analysis.

All fluid for chemical testing should be separated from cellular elements and debris as soon as possible to prevent distortion of chemical constituents by cellular metabolism or disintegration. This can be performed using centrifugation or filtration. Filtration is recommended for FLM methods to prevent loss of the phospholipids.

■ ■ ● Color and Appearance

Normal amniotic fluid is colorless and may exhibit slight to moderate turbidity from cellular debris, particularly in later stages of fetal development. Blood-streaked fluid may be present as the result of a traumatic tap, abdominal trauma, or intra-amniotic hemorrhage. The source of the blood (maternal or fetal) can be determined using the Kleihauer-Betke test for fetal hemoglobin and is important for further case management.

The presence of bilirubin gives the fluid a yellow color and is indicative of red blood cell destruction resulting from HDN. *Meconium*, which is usually defined as a newborn's first bowel movement, may be present in the amniotic fluid as the result of fetal intestinal secretions. It produces a dark green color. Fetal aspiration of meconium during fetal swallowing is a concern when increased amounts are present in the fluid. A very dark red-brown fluid is associated with fetal death.

Tests for Fetal Distress

Hemolytic Disease of the Newborn

The oldest routinely performed laboratory test on amniotic fluid evaluates the severity of the fetal anemia produced by

Amniotic Fluid Color		
Color	Significance	
Colorless	Normal	
Blood-streaked	Traumatic tap, abdominal trauma, intra-amniotic hemorrhage	
Yellow	Hemolytic disease of the newborn (bilirubin)	
Dark green Dark red-brown	Meconium Fetal death	

HDN. The incidence of this disease has been decreasing rapidly since the development of methods to prevent anti-Rh antibody production in postpartum mothers. However, antibodies against other red cell antigens are also capable of producing HDN, and immunization of Rh-negative mothers may not be effective or even performed in all cases. Initial exposure to foreign red cell antigens occurs during gestation and delivery of the placenta when fetal red blood cells enter into the maternal circulation and stimulate the mother to produce antibodies to the antigen. When these antibodies present in the maternal circulation cross the placenta into the fetal circulation and bind to the antigen on the fetal cells, the cells are destroyed. The destruction of fetal red blood cells results in the appearance of the red blood cell degradation product, unconjugated bilirubin, in the amniotic fluid. By measuring the amount of bilirubin in the fluid, the extent of hemolysis taking place may be determined, and the danger this anemia presents to the fetus may be assessed (Fig. 14-2).

The measurement of amniotic fluid bilirubin is performed by spectrophotometric analysis. As illustrated in Figure 14-3, the optical density (OD) of the fluid is measured in intervals between 365 nm and 550 nm and the readings plotted on semilogarithmic graph paper. In normal fluid, the OD is highest at 365 nm and decreases linearly to 550 nm, illustrated by a straight line. When bilirubin is present, a rise in OD is seen at 450 nm because this is the wavelength of maximum bilirubin absorption. The difference between the OD of the theoretic baseline and the OD at 450 nm represents the amniotic fluid bilirubin concentration. This difference in OD, referred to as the absorbance difference at 450 nm (Δ A450), is then plotted on a Liley graph to determine the severity of the hemolytic disease (Fig. 14-4).

Notice that the Liley graph plots the $\Delta A450$ against gestational age and is divided into three zones that represent the extent of hemolytic severity. Values falling in zone I indicate no more than a mildly affected fetus; those in zone II require careful monitoring, whereas a value in zone III suggests a severely affected fetus. Intervention through induction of labor or intrauterine exchange transfusion must be considered when a $\Delta A450$ is plotted in zone III.

As mentioned, specimens must be protected from light at all times. Markedly decreased values will be obtained with as little as 30 minutes of exposure to light. Care must be taken to ensure that contamination of the fluid by cells, hemoglobin, meconium, or other debris does not interfere with the spectrophotometric analysis. Specimens should be immediately centrifuged to remove particulate interference. Maximum absorbance of oxyhemoglobin occurs at 410 nm and can interfere with the bilirubin absorption peak (see Fig. 14-3). This interference can be removed by extraction with chloroform if necessary.7 A control may be prepared by diluting commercial chemistry control sera 1 to 10 with normal saline and treating it in the same manner as the patient specimen. Bilirubin and protein levels approximate those in amniotic fluid and can be varied by using low or high control sera.8

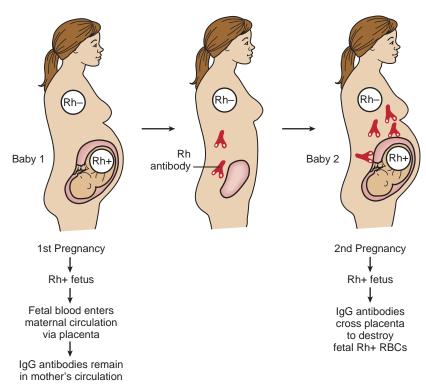


Figure 14–2 Rh antibodies crossing the placenta

Neural Tube Defects

Increased levels of alpha-fetoprotein (AFP) in both the maternal circulation and the amniotic fluid can be indicative of fetal neural tube defects, such as anencephaly and spina bifida. AFP is the major protein produced by the fetal liver during early gestation (prior to 18 weeks). It is found in the maternal serum due to the combined fetal-maternal circulations and in the amniotic fluid from diffusion and excretion of fetal urine. Increased levels are found in the maternal serum and amniotic fluid when the skin fails to close over the neural tissue, as occurs in anencephaly and spina bifida.

Measurement of amniotic fluid AFP levels is indicated when maternal serum levels are elevated or a family history of previous neural tube defects exists. The possibility of a multi-

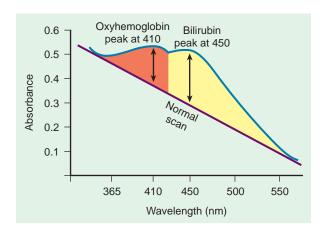


Figure 14–3 Spectrophotometric bilirubin scan showing bilirubin and oxyhemoglobin peaks.

ple pregnancy also must be investigated when serum levels are elevated. Normal values are based on the week of gestational age, as the fetus produces maximal AFP between 12 and 15 weeks' gestation, after which levels in amniotic fluid begin to decline. Both serum and amniotic fluid AFP levels are reported in terms of multiples of the median (MoM). The median is the laboratory's reference level for a given week of gestation. A value two times the median value is considered abnormal (greater than two MoM) for both maternal serum and amniotic fluid. Testing for AFP has been automated by the Access Immunoassay System (Beckman Coulter, Inc., Fullerton, Calif.).

Elevated amniotic fluid AFP levels are followed by measurement of amniotic acetylcholinesterase (AChE). The test is more specific for neural tube disorders than AFP, provided it is not performed on a bloody specimen, because blood contains AChE.³

■■● Tests for Fetal Maturity

Fetal distress, whether caused by HDN or other conditions, forces the obstetrician to consider a preterm delivery. At this point, fetal maturity must be assessed.

Fetal Lung Maturity

Respiratory distress syndrome (RDS) is the most frequent complication of early delivery and is a cause of morbidity and mortality in the premature infant. This disease is caused by a lack of lung surfactant, a substance that normally appears in mature lungs and allows the alveoli (air sacs of the lung) to remain open throughout the normal cycle of inhalation and

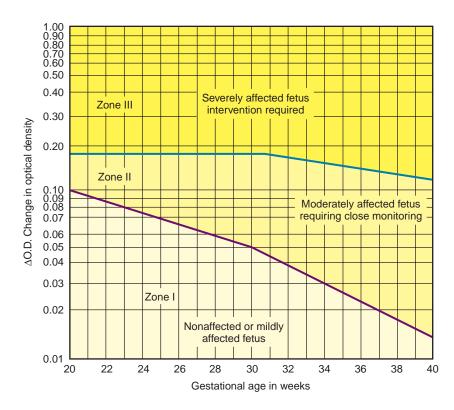


Figure 14-4 Example of a Liley graph.

exhalation. Surfactant keeps the alveoli from collapsing by decreasing surface tension and allows them to inflate with air more easily. Therefore, laboratory tests must be performed to determine the maturity of the fetal lungs. Several laboratory tests are available to measure FLM.

Lecithin-Sphingomyelin Ratio

The reference method to which tests of FLM are compared is the *lecithin-sphingomyelin* (L/S) *ratio*. Lecithin is the primary component of the *surfactants* (phospholipids, neutral lipids, and proteins) that make up the alveolar lining and account for alveolar stability.

Lecithin is produced at a relatively low and constant rate until the 35th week of gestation, at which time a noticeable increase in its production occurs, resulting in the stabilization of the fetal lung alveoli. Sphingomyelin is a lipid that is produced at a constant rate after about 26 weeks' gestation; therefore, it can serve as a control on which to base the rise in lecithin. Both lecithin and sphingomyelin appear in the amniotic fluid in amounts proportional to their concentrations in the fetus. 9 Prior to 35 weeks' gestation, the L/S ratio is usually less than 1.6 because large amounts of lecithin are not being produced at this time. It will rise to 2.0 or higher when lecithin production increases to prevent alveolar collapse. Therefore, when the L/S ratio reaches 2.0, a preterm delivery is usually considered to be a relatively safe procedure. Falsely elevated results are encountered in fluid contaminated with blood or meconium because both these substances contain lecithin and sphingomyelin.

Quantitative measurement of lecithin and sphingomyelin is performed using thin-layer chromatography. The procedure

is labor intensive and subject to high coefficients of variation. Many laboratories have replaced the L/S ratio with the more cost-effective *phosphatidyl glycerol* immunoassays, fluorescence polarization, and *lamellar body* density procedures.¹⁰

Amniostat-FLM

The presence of another lung surface lipid, phosphatidyl glycerol, is also essential for adequate lung maturity. The production of phosphatidyl glycerol normally parallels that of lecithin, but its production is delayed in cases of maternal diabetes. In this circumstance, respiratory distress occurs in the presence of an L/S ratio of 2.0. Therefore, a thin-layer chromatography lung profile must include lecithin, sphingomyelin, and phosphatidyl glycerol to provide an accurate measurement.¹¹

Development of an immunologic agglutination test for phosphatidyl glycerol has provided a more rapid method for assessment of fetal maturity that does not require a laboratory to be equipped to perform thin-layer chromatography. The Aminostat-FLM (Irving Scientific, Santa Ana, Calif.) uses antisera specific for phosphatidyl glycerol and is not affected by specimen contamination with blood and meconium. ¹² Studies have shown good correlation with thin-layer chromatography but with a slightly higher incidence of false-negative results that may need to be followed up with further testing. ^{13,14}

Foam Stability

Until the development of biochemical techniques to measure the individual lung-surface lipid concentrations, a mechanical screening test, called the "foam" or "shake" test, was used to determine their presence. Because it can be performed at the bedside or in the laboratory, the test is still in use. Amniotic fluid is mixed with 95% ethanol, shaken for 15 seconds, and allowed to sit undisturbed for 15 minutes. At the end of this time, the surface of the fluid is observed for the presence of a continuous line of bubbles around the outside edge. The presence of bubbles indicates that a sufficient amount of phospholipid is available to reduce the surface tension of the fluid even in the presence of alcohol, an antifoaming agent.

A modification of the foam test uses 0.5 mL of amniotic fluid added to increasing amounts of 95% ethanol, providing a gradient of ethanol/fluid ratios ranging from 0.42 mL to 0.55 mL in 0.01-mL increments, which can be used to provide a semiquantitative measure of the amount of surfactant present. A value of 47 or higher indicates FLM. The Foam Stability Index has shown good correlation with the L/S ratio and tests for phosphatidyl glycerol. The test cannot be used with contaminated amniotic fluid because blood and meconium also reduce surface tension.

Microviscosity: Fluorescence Polarization Assay

The presence of phospholipids decreases the microviscosity of the amniotic fluid. This change in microviscosity can be measured using the principle of fluorescence polarization employed by the Abbott TDx analyzer with the TDx/TDxFLx FLM II Assay System (Abbott Laboratories, Abbott Park, Ill.). The TDx/TDxFLx Fetal Lung Maturity II (FLMII) assay is a reagent system for the quantitative measurement of the ratio of surfactant to albumin in amniotic fluid for assessment of lung maturity of the fetus. 5 This assay measures the polarization of a fluorescent dye that combines with both surfactants and albumin. Dye bound to surfactant has a longer fluorescence lifetime and exhibits low polarization, whereas dye bound to albumin has a decreased fluorescence lifetime and has high polarization. Albumin is used as an internal standard in the same manner as sphingomyelin because it remains at a constant level throughout gestation. The recorded changes in polarization produce a surfactant/albumin ratio expressed in milligrams surfactant to grams albumin that is compared with a fetal lung maturity II assay calibration standard curve that includes phosphatidyl glycerol and ranges from 0 to 160 mg/g. Fetal Lung Maturity II Calibrators of known surfactant/albumin ratio are run and the resulting standard curve is

PROCEDURE



Foam Shake Test Procedure

- 1. Mix equal parts of amniotic fluid with 95% ethanol.
- 2. Vigorously shake for 15 seconds.
- 3. Allow to sit undisturbed for 15 minutes.
- **4.** Observe for the presence of a continuous line of bubbles around the outside edge.

PROCEDURE



Procedure for Foam Stability Index

- 1. Add 0.5 mL of amniotic fluid to tubes containing increasing amounts of 95% ethanol ranging from 0.42 to 0.55 mL in 0.01-mL increments.
- 2. Vigorously shake for 15 seconds.
- 3. Allow to sit undisturbed for 15 minutes.
- **4.** Observe for the presence of a continuous line of bubbles around the outside edge.
- 5. Values ≥47 indicate fetal lung maturity.

stored in memory. Sample results are calculated from the stored standard curve using polarization values generated for each sample. A value of 55 mg surfactant per gram albumin or greater provides a conservative indicator of FLM and lower values may be considered. Immature results with the FLM II assay are less than or equal to 39 mg/g. Results between 40 mg/g and 54 mg/g cannot be declared "mature" or "immature" and must be evaluated with caution. The TDx–FLM II test value correlates well with a L/S ratio of 2.0 and has few falsely mature results, making it an excellent screening tool. Sequential L/S testing is recommended when the results of the TDx–FLM II suggest fetal lung immaturity. An accurate gestational age is an important consideration in interpreting the results.

The test requires at least 1.0 mL of amniotic fluid. Fluid should be filtered rather than centrifuged prior to examination to prevent sedimentation of the lipids and reporting a falsely decreased result. Specimens contaminated with blood, meconium, suspected maternal urine, and visibly icteric samples should not be used.⁵

Lamellar Bodies and Optical Density

Lamellar bodies are lamellated phospholipids that represent a storage form of surfactant. The surfactants responsible for FLM are produced and secreted by the type II pneumocytes of the fetal lung and stored in the form of structures termed lamellar bodies at about 20 weeks of gestation. The lamellar bodies enter the alveolar spaces to provide surfactant and also enter the amniotic fluid at about 26 weeks of gestation. As the fetal lung matures, increased lamellar body production is reflected by an increase in amniotic fluid phospholipids and the L/S ratio. ¹⁶ Therefore, the number of lamellar bodies present in the amniotic fluid correlates with the amount of phospholipid present in the fetal lungs.

The presence of lamellar bodies increases the OD of the amniotic fluid. Specimens are centrifuged at 2000 g for 10 minutes and examined using a wavelength of 650 nm, which rules out interference from hemoglobin but not other contaminants, such as meconium. An OD of 0.150 has been shown to correlate well with an L/S ratio of greater than or equal to 2.0 and the presence of phosphatidyl glycerol.¹⁷

242 CHAPTER 14 • Amniotic Fluid

Lamellar body diameter is similar to that of small platelets; therefore, lamellar body counts (LBCs) can be obtained rapidly with use of the platelet channel of hematology analyzers. The various instruments use different principles to identify platelets and are usually not invalidated by the presence of lysed blood, bilirubin, or meconium. LBCs performed on amniotic fluid stored at 4°C are stable for up to 10 days. 18

Lamellar bodies can be counted using resistance-pulse counting, such as that employed by Coulter cell-counting instruments (Beckman Coulter, Inc., Fullerton, Calif.). Ranging in size from 1.7 to 7.3 fL, lamellar bodies can be counted using the platelet channel. 19 This technique is easily performed; however, samples must be free of particle contamination such as meconium and blood. A count of 32,000 or more particles per microliter represents adequate FLM.²⁰ The ADVIA 120 hematology system (Siemens Medical Solutions Diagnostics, Diagnostics Division, Tarrytown, N.Y.) measures two light-scatter angles of particles as they pass through a laser beam and identifies particles based on their cell volume indices and refractive index. The LBC count is the sum of all platelet-sized particles measured in the platelet channel (calculated LBC). A count of 35,400 or more particles per microliter indicates FLM.²¹ Sysmex XE-2100 (Sysmex, Mundelein, Ill.) technology simultaneously detects direct current and radiofrequency impedance thought to reflect intracellular changes. Cell-dyn 3500 (Abbott Laboratories, Abbott Park, Ill.) combines optical scatter and impedance. Because the various hematology analyzers count lamellar bodies differently and require different specimen preparation, cutoff values for FLM vary, making it necessary to establish analyzer-specific LBC clinical decision limits. 18

References

- Ross, MG, Brace, RA, and the NIH Workshop Participants, National Institute of Child Health and Development Conference summary: Amniotic fluid biology—basic and clinical aspects. The Journal of Maternal-Fetal Medicine 10:2-19, 2001.
- 2. Weiss, RR, et al: Amniotic fluid uric acid and creatinine as measures of fetal maturity. Obstet Gynecol 44(2):208-214, 1974.
- Wenk, RE, and Rosenbaum, JM: Examination of amniotic fluid. In Henry, JB (ed): Clinical Diagnosis and Management by Laboratory Methods. WB Saunders, Philadelphia, 1996.
- 4. Heron, HJ: The use of the Fern test to differentiate amniotic fluid from urine. Triangle Jul:20:60-62, 1963.
- TDx/TDxFLx Fetal Lung Maturity II (FLM II) package insert. Reference 7A76. Abbott Laboratories, Diagnostics Division, Abbott Park, Ill., April 2003.
- 6. Liley, AW: Liquor amnii analysis in the management of the pregnancy complicated by Rhesus sensitization. Am J Obstet Gynecol 82:1359, 1961.
- 7. Spinnato, JA, et al: Amniotic bilirubin and fetal hemolytic disease. Am J Obstet Gynecol 165(4):1030-1035, 1991.
- 8. McDonald, OL, and Watts, MT: Use of commercially prepared control sera as quality control materials for spectrophotometric bilirubin determinations in amniotic fluid. Am J Clin Pathol 84(4):513-517, 1985.
- 9. Gluck, L, et al: Diagnosis of the respiratory distress syndrome by amniocentesis. Am J Obstet Gynecol 109(3):440-445, 1971.
- 10. Dubin, SB: Assessment of FLM: Practice parameter. Am J Clin Pathol 110:723-732, 1998.

- 11. Kulovich, MV, Hallman, MB, and Gluck, L: The lung profile: Normal pregnancy. Am J Obstet Gynecol 135:57-60, 1979.
- 12. Eisenbrey, AB, et al: Phosphatidyl glycerol in amniotic fluid: Comparison of an "ultrasensitive" immunologic assay with TLC and enzymatic assay. Am J Clin Pathol 91(3):293-297, 1989.
- 13. Chapman, JF: Current methods for evaluating FLM. Lab Med 17(10):597-602, 1986.
- 14. Saad, SA, et al: The reliability and clinical use of a rapid phosphatidyl glycerol assay in normal and diabetic pregnancies. Am J Obstet Gynecol 157(6):1516-1520, 1987.
- 15. Winn-McMillan, T, and Karon, BS: Comparison of the TDx-FLM II and lecithin to sphingomyelin ratio assays in predicting fetal lung maturity. Am J Obstet Gynecol 193,778-782, 2005.
- 16. Khazardoost, S, et al: Amniotic fluid lamellar body count and its sensitivity and specificity in evaluating of fetal lung maturity. J Obstet Gynaecol 25(3):257-259, 2005.
- 17. Sbarra, AJ, et al: Correlation of amniotic fluid optical density at 650 nm and lecithin/sphingomyelin ratios. Obstet Gynecol 48:613, 1976.
- 18. Szallasi, A, Gronowski, AM, and Eby, CS: Lamellar body count in amniotic fluid: A comparative study of four different hematology analyzers. Clin Chem 49:994-997, 2003.
- 19. Ashwood, ER, et al: Measuring the number of lamellar body particles in amniotic fluid. Obstet Gynecol 75:289-292, 1990.
- Fakhoury, G, et al: Lamellar body concentrations and the prediction of fetal pulmonary maturity. Am J Obstet Gynecol 170:72, 1994.
- 21. Chapman, JF, et al: Evaluation of two-dimensional cytometric lamellar body counts on the ADVIA®120 hematology system for estimation of fetal lung maturation. Clin Chim Acta 340(1-2):85-92, 2004.

QUESTIONS

- 1. Which of the following is not a function of amniotic fluid?
 - A. Allow movement of the fetus
 - B. Carbon dioxide and oxygen exchange
 - C. Protect fetus from extreme temperature changes
 - D. Protective cushion for the fetus
- **2.** What is the primary cause of the normal increase in amniotic fluid as a pregnancy progresses?
 - A. Fetal cell metabolism
 - B. Fetal swallowing
 - C. Fetal urine
 - D. Transfer of water across the placenta
- 3. Which of the following is not a reason for decreased amounts of amniotic fluid?
 - A. Fetus fails to begin swallowing
 - B. Increased fetal swallowing
 - C. Membrane leakage
 - D. Urinary tract defects
- **4.** Why might a creatinine level be requested on an amniotic fluid?
 - A. Detect oligohydramnios
 - B. Detect polyhydramnios
 - C. Differentiate amniotic fluid from maternal urine
 - D. Evaluate lung maturity

- **5.** Amniotic fluid specimens are placed in ambercolored tubes prior to sending them to the laboratory to prevent the destruction of:
 - A. Alpha fetoprotein
 - B. Bilirubin
 - C. Cells for cytogenetics
 - D. Lecithin
- **6.** How are specimens for FLM testing delivered to and stored in the laboratory?
 - A. Delivered on ice and refrigerated or frozen
 - B. Immediately centrifuged
 - C. Kept at room temperature
 - D. Delivered in a vacuum tube
- 7. Why are amniotic specimens for cytogenetic analysis incubated at 37°C prior to analysis?
 - A. To detect the presence of meconium
 - B. To differentiate amniotic fluid from urine
 - C. To prevent photo-oxidation of bilirubin to biliverdin
 - D. To prolong fetal cell viability and integrity
- **8.** Filtration of amniotic fluid is required to avoid decreased values in the test results for:
 - A. Bilirubin
 - B. Fetal cells
 - C. Phospholipids
 - D. Urea
- **9.** Match the following colors in amniotic fluid with their significance.
 - ___A. Colorless
- 1. Fetal death
- ___B. Dark green
- 2. Normal
- C. Red-brown
- 3. Presence of bilirubin
- ___D. Yellow
- 4. Presence of meconium
- **10.** A significant rise in the OD of amniotic fluid at 450 nm indicates the presence of which analyte?
 - A. Bilirubin
 - B. Lecithin
 - C. Oxyhemoglobin
 - D. Sphingomyelin
- 11. Plotting the amniotic fluid OD on a Liley graph represents the severity of hemolytic disease of the newborn. A value that is plotted in zone II indicates what condition of the fetus?
 - A. No hemolysis
 - B. Mildly affected fetus
 - C. Moderately affected fetus that requires close monitoring
 - D. Severely affected fetus that requires intervention

- **12**. The presence of a fetal neural tube disorder may be detected by:
 - A. Increased amniotic fluid bilirubin
 - B. Increased maternal serum alpha fetoprotein
 - C. Decreased amniotic fluid phosphatidyl glycerol
 - D. Decreased maternal serum acetycholinesterase
- **13**. *True or False*: An AFP MoM value greater than two times the median value is considered an indication of a neural tube disorder.
- **14.** When severe HDN is present, which of the following tests on the amniotic fluid would the physician *not* order to determine whether the fetal lungs are mature enough to withstand a premature delivery?
 - A. AFP levels
 - B. Foam stability index
 - C. Lecithin/sphingomyelin ratio
 - D. Phosphatidyl glycerol detection
- **15**. The foam or shake test is a screening test for which amniotic fluid substance?
 - A. Bilirubin
 - B. Lecithin
 - C. Alpha fetoprotein
 - D. Creatinine
- **16**. *True or False*: Prior to 35 weeks' gestation, the normal L/S ratio is less than 1.6.
- **17**. When performing an L/S ratio by thin-layer chromatography, a mature fetal lung will show:
 - A. Sphingomyelin twice as concentrated as lecithin
 - B. No sphingomyelin
 - C. Lecithin twice as concentrated as sphingomyelin
 - D. Equal concentrations of lecithin and sphingomyelin
- **18**. *True or False*: Phosphatidyl glycerol is present with an L/S ratio of 1.1.
- **19**. A rapid test for FLM that does not require performance of thin-layer chromatography is:
 - A. AFP levels
 - B. Amniotic acetylcholinesterase
 - C. Aminostat-FLM
 - D. Bilirubin scan
- **20.** Does the failure to produce bubbles in the Foam Stability Index indicate increased or decreased lecithin?
 - A. Increased
 - B. Decreased
- **21**. Microviscosity of amniotic fluid is measured by:
 - A. Thin-layer chromatography
 - B. Immunologic agglutination
 - C. Spectrophotometer
 - D. Fluorescence polarization

244 CHAPTER 14 • Amniotic Fluid

Continued

- **22.** The presence of phosphatidyl glycerol in amniotic fluid fetal lung maturity tests must be confirmed when:
 - A. Hemolytic disease of the newborn is present
 - B. The mother has maternal diabetes
 - C. Amniotic fluid is contaminated by hemoglobin
 - D. Neural tube disorder is suspected
- **23.** Match the following principles with the appropriate FLM test.

Principle FLM Test ___A. Immunologic agglutination test ___B. Uses albumin as the internal standard ___C. Uses the platelet channel on a hematology instrument ___D. Uses sphingomyelin as an internal standard 4. True or False: An L/S ratio of 2.0 correlates with a cure.

- **24**. *True or False*: An L/S ratio of 2.0 correlates with a surfactant/albumin ratio of 39 mg/g.
- **25.** A lamellar body count of 50,000 correlates with:
 - A. Absent phosphatidyl glycerol and TDx-FLM II ratio of 39
 - B. L/S ratio of 1.5 and absent phosphatidyl glycerol
 - C. OD at 650 nm of 1.010 and an L/S ratio of 1.1
 - D. OD at 650 nm of 0.150 and an L/S ratio of 2.0
- **26.** Which test for FLM is least affected by contamination with hemoglobin and meconium?
 - A. Amniostat-FLM
 - B. Foam Stability
 - C. Lamellar Bodies Count
 - D. TDx-FLM II

Case Studies and Clinical Situations

- 1. Amniocentesis is performed on a woman believed to be in approximately the 31st week of gestation. This is the second pregnancy for this Rh-negative, woman with diabetes. Spectrophotometric analysis of the fluid shows a $\Delta A450$ of 0.3.
 - a. Based on the Liley graph, should the physician consider inducing labor?
 - b. What else must the physician consider prior to inducing labor?

The physician decides to induce labor based on a positive Aminostat-FLM.

- c. What information did this test provide for the physician?
- d. Why did the physician prefer an Aminostat-FLM over an L/S ratio in this situation?
- **2.** Amniocentesis is performed following a maternal serum AFP level of 2.2 MoM at 15 weeks' gestation.
 - a. What fetal condition is suspected?
 - b. If the amniotic fluid AFP is 2.5 MoM, what additional test could be performed?
 - c. In what situation would this additional test not be performed?
- **3.** If the amount of fluorescence polarization in an amniotic fluid is decreased, does this represent increased or decreased lecithin?
- **4.** Amniotic fluid for FLM testing is centrifuged for 10 minutes at 5000 g. How will this affect the test results?
- **5.** How might a dark green amniotic fluid affect the results of the following tests?
 - a. Foam Stability Index
 - b. L/S ratio
 - c. Aminostat-FLM
 - d. OD₆₅₀
- **6.** How might a blood-streaked amniotic fluid affect the results of the following tests?
 - a. L/S ratio
 - b. AChE
 - c. Bilirubin analysis
 - d. Aminostat-FLM
- 7. Amniocentesis is performed on a woman whose last two pregnancies resulted in stillbirths due to hemolytic disease of the newborn. A screening test performed at the hospital is positive for bilirubin, and the specimen is sent to a reference laboratory for a bilirubin scan. Physicians are concerned when the report comes back negative. What factors would be considered in evaluating this result.
 - a. Correct specimen was sent
 - b. Specimen was refrigerated
 - c. Specimen was exposed to light
 - d. Specimen reached the reference lab within 30 minutes













Fecal Analysis

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

- 1 Describe the normal composition of feces.
- 2 Differentiate between secretory and osmotic diarrhea.
- 3 List three causes of diarrhea and steatorrhea.
- **4** Differentiate malabsorption from maldigestion syndromes and name a test that distinguishes the two conditions.
- 5 Instruct patients in the collection of random and quantitative stool specimens.
- **6** State a pathogenic and a nonpathogenic cause for stools that are red, black, and pale yellow.
- 7 State the significance of bulky, ribbon-like, and mucus-containing stools.
- **8** State the significance of increased neutrophils in a stool specimen.
- 9 Describe a positive microscopic examination for muscle fibers.

- 10 Name the fecal fats stained by Sudan III, and give the conditions under which they will stain.
- 11 Describe and interpret the microscopic results that are seen when a specimen from a patient with steatorrhea is stained with Sudan III.
- 12 Explain the principle of the guaiac test for occult blood and the reasons that guaiac is the reagent of choice.
- 13 Instruct a patient in the collection of specimens for occult blood, including providing an explanation of dietary restrictions.
- 14 Briefly describe a chemical screening test performed on feces for each of the following: fetal hemoglobin, pancreatic insufficiency, and carbohydrate intolerance.

KEY TERMS

constipation malabsorption maldigestion occult blood
osmotic diarrhea
pancreatic insufficiency

secretory diarrhea steatorrhea

In the minds of most laboratory personnel, analysis of fecal specimens fits into the category of a "necessary evil." However, as an end product of body metabolism, feces do provide valuable diagnostic information. Routine fecal examination includes macroscopic, microscopic, and chemical analyses for the early detection of gastrointestinal (GI) bleeding, liver and biliary duct disorders, *maldigestion/malabsorption* syndromes, inflammation, and causes of diarrhea and steatorrhea. Of equal diagnostic value is the detection and identification of pathogenic bacteria and parasites; however,

these procedures are best covered in a microbiology textbook and are not discussed here.

Physiology

The normal fecal specimen contains bacteria, cellulose, and other undigested foodstuffs, gastrointestinal secretions, bile pigments, cells from the intestinal walls, electrolytes, and water. Many species of bacteria make up the normal flora of the intestines. Bacterial metabolism produces the strong odor

246 CHAPTER 15 • Fecal Analysis

associated with feces and intestinal gas (*flatus*). Carbohydrates, especially oligosaccharides, that are resistant to digestion pass through the upper intestine unchanged but are metabolized by bacteria in the lower intestine, producing large amounts of flatus. Excessive gas production also occurs in lactose-intolerant individuals when the intestinal bacteria metabolize the lactose from consumed milk or lactose-containing substances.

Although digestion of ingested proteins, carbohydrates, and fats takes place throughout the *alimentary tract*, the small intestine is the primary site for the final breakdown and reabsorption of these compounds. Digestive enzymes secreted into the small intestine by the pancreas include trypsin, chymotrypsin, amino peptidase, and lipase. Bile salts provided by the liver aid in the digestion of fats. A deficiency in any of these substances causes the inability to digest and, therefore, to reabsorb certain foods. Excess undigested or unreabsorbed material then appear in the feces, and the patient exhibits symptoms of maldigestion and malabsorption. As shown in Figure 15-1, approximately 9000 mL of ingested fluid, saliva, gastric, liver, pancreatic, and intestinal secretions enter the digestive tract each day. Under normal conditions, only

between 500 to 1500 mL of this fluid reaches the large intestine, and only about 150 mL is excreted in the feces. Water and electrolytes are readily absorbed in both the small and large intestines, resulting in a fecal electrolyte content that is similar to that of plasma.

The large intestine is capable of absorbing approximately 3000 mL of water. When the amount of water reaching the large intestine exceeds this amount, it is excreted with the solid fecal material, producing *diarrhea*. *Constipation*, on the other hand, provides time for additional water to be reabsorbed from the fecal material, producing small, hard *stools*.

Diarrhea

Diarrhea is defined as an increase in daily stool weight above 200 g with increased liquidity and frequency of more than three times per day. Diarrhea classification can be based on four factors: duration of the illness, mechanism, severity, and stool characteristics. Diarrhea lasting less than 4 weeks is defined as acute, and diarrhea persisting for more than 4 weeks is termed chronic diarrhea.

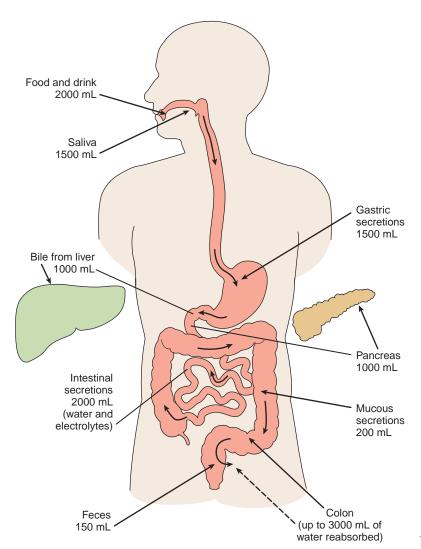


Figure 15–1 Fluid regulation in the gastrointestinal tract.

The major mechanisms of diarrhea are secretory, osmotic, and altered motility. The laboratory tests used to differentiate these mechanisms are fecal electrolytes (fecal sodium, fecal potassium), fecal osmolality, and stool pH. The total fecal osmolarity is close to the serum osmolality (290 mOsm/kg). The fecal sodium and fecal potassium results are used to calculate the fecal osmotic gap. The fecal osmotic gap is calculated as follows:

Osmotic gap = 290 - [2 (fecal sodium + fecal potassium)]

The osmotic gap in all forms of osmotic diarrhea is greater than 50 mOsm/kg and less than 50 mOsm/kg in secretory diarrhea. Electrolytes are increased in secretory diarrhea and neglible in osmotic diarrhea. A fecal fluid pH of less than 5.6 indicates a malabsorption of sugars, causing an osmotic diarrhea.

Secretory Diarrhea

Bacterial, viral, and protozoan infections produce increased secretion of water and electrolytes, which override the reabsorptive ability of the large intestine (secretory diarrhea). Enterotoxin-producing organisms such as Escherichia coli, Clostridium, Vibrio cholerae, Salmonella, Shigella, Staphylococcus, Campylobacter, protozoa, and parasites such as Cryptosporidium can stimulate these water and electrolyte secretions. Other causes of secretory diarrhea are drugs, stimulant laxatives, hormones, inflammatory bowel disease (Crohn disease, ulcerative colitis, lymphocytic colitis, diverticulitis), endocrine disorders (hyperthyroidism, Zollinger-Ellison syndrome, vipoma), neoplasms, and collagen vascular disease.

Osmotic Diarrhea

Incomplete breakdown or reabsorption of food presents increased fecal material to the large intestine, resulting in the retention of water and electrolytes in the large intestine (osmotic diarrhea), which in turn results in excessive watery stool. Maldigestion (the impaired digestion of food) and malabsorption (the impaired absorption of nutrients by the intestine) contribute to osmotic diarrhea. The presence of unabsorbable solute increases the stool osmolality and the concentration of electrolytes is lower, resulting in an increased osmotic gap. Causes of osmotic diarrhea include disaccharidase deficiency (lactose intolerance), malabsorption (celiac sprue), poorly absorbed sugars (lactose, sorbitol, mannitol), laxatives, magnesium-containing antacids, amebiasis, and antibiotic administration. Laboratory testing of feces is frequently performed to aid in determining the cause of diarrhea (Table 15-1).

Altered Motility

Altered motility describes conditions of enhanced motility (hypermotility) or slow motility (constipation). Both can be seen in irritable bowel syndrome (IBS), which is a functional

Table 15-1 Common Fecal Tests for Diarrhea	
Secretory	Osmotic
Stool cultures	Microscopic fecal fats
Ova and parasite examinations	Muscle fiber detection
Rotavirus immunoassay	Qualitative fecal fats
Fecal leukocytes	Trypsin screening Microscopic fecal fats Muscle fiber detection Quantitative fecal fats Clinitest D-xylose tolerance test Lactose tolerance test Fecal electrolytes Stool pH Fecal osmolality

disorder in which the nerves and muscles of the bowel are extra sensitive, causing cramping, bloating, flatus, diarrhea, and constipation. IBS can be triggered by food, chemicals, emotional stress, and exercise.

Rapid (accelerated) gastric emptying (RGE) dumping syndrome describes hypermotility of the stomach and the shortened gastric emptying half-time, which causes the small intestine to fill too quickly with undigested food from the stomach. It is the hallmark of early dumping syndrome (EDS). Healthy individuals have a gastric emptying half-time range of 35 to 100 minutes, which varies with age and gender. A gastric emptying time of less than 35 minutes is considered RGE.1 This can be caused by disturbances in the gastric reservoir or in the transporting function. Alterations in the motor functions of the stomach result in the accumulation of large amounts of osmotically active solids and liquids to be transported into the small intestine. Normal gastric emptying is controlled by fundic tone, duodenal feedback, and GI hormones. These are altered after gastric surgery, resulting in clinically significant dumping syndrome in approximately 10% of patients.²

RGE can be divided into early dumping and late dumping depending upon how soon after a meal the symptoms occur. EDS symptoms begin 10 to 30 minutes following meal ingestion.² Symptoms include nausea, vomiting, bloating, cramping, diarrhea, dizziness, and fatigue. Late dumping occurs 2 to 3 hours after a meal and is characterized by weakness, sweating, and dizziness.¹ Hypoglycemia is often a complication of dumping syndrome. The main causes of dumping syndrome include gastrectomy, gastric bypass surgery, postvagotomy status, Zollinger-Ellison syndrome, duodenal ulcer disease, and diabetes mellitus.¹

248 CHAPTER 15 • Fecal Analysis

Steatorrhea

Detection of steatorrhea is useful for the diagnosis of pancreatic insufficiency and small bowel disorders that cause malabsorption. Absence of bile salts that assist pancreatic lipase in the breakdown and subsequent reabsorption of triglycerides produces an increase in stool fat (steatorrhea) that exceeds 6 g per day. Likewise, pancreatic disorders, including cystic fibrosis, chronic pancreatitis, and carcinoma that decrease the production of pancreatic enzymes, are also associated with steatorrhea. Steatorrhea may be present in both maldigestion and malabsorption conditions and can be distinguished by the D-xylose test. D-xylose is a sugar that does not need to be digested but does need to be absorbed to be present in the urine. If urine D-xylose is low, the resulting steatorrhea would indicate a malabsorption condition. Malabsorption causes include bacterial overgrowth, intestinal resection, celiac disease, tropical sprue, lymphoma, Whipple disease, Giardia lamblia infestation, Crohn disease, and intestinal ischemia. A normal D-xylose test indicates pancreatitis.

Specimen Collection

Collection of a fecal specimen, frequently called a stool specimen, is not an easy task for patients. Detailed instructions and appropriate containers should be provided.

Patients should be instructed to collect the specimen in a clean container, such as a bedpan or disposable container, and transfer the specimen to the laboratory container. Patients should understand that the specimen must not be contaminated with urine or toilet water, which may contain chemical disinfectants. Some kits provided for the collection of specimens to be screened for occult blood contain paper that can be floated in the toilet bowl to collect the specimen. This method should only be used when collecting specimens to be tested using the kit in which they are included. Containers that contain preservatives for ova and parasites must not be used to collect specimens for other tests.

Random specimens suitable for qualitative testing for blood and microscopic examination for leukocytes, muscle fibers, and fecal fats are usually collected in plastic or glass containers with screw-capped tops similar to those used for urine specimens. Material collected on a physician's glove and samples applied to filter paper in occult blood testing kits are also received.

For quantitative testing, such as for fecal fats, timed specimens are required. Because of the variability of bowel habits and the transit time required for food to pass through the digestive tract, the most representative sample is a 3-day collection. These specimens are frequently collected in paint cans to accommodate the specimen quantity and facilitate emulsification prior to testing. Care must be taken when opening any fecal specimen to slowly release gas that has accumulated within the container. Also, patients must be cautioned not to contaminate the outside of the container.

■■● Macroscopic Screening

The first indication of gastrointestinal disturbances can often be provided by changes in the brown color and formed consistency of the normal stool. Of course, the appearance of abnormal fecal color may also be caused by the ingestion of highly pigmented foods and medications, so a differentiation must be made between this and a possible pathologic cause.

Color

The brown color of the feces results from intestinal oxidation of stercobilinogen to urobilin. As discussed in Chapter 5, conjugated bilirubin formed in the degradation of hemoglobin passes through the bile duct to the small intestine where intestinal bacteria convert it to urobilinogen and stercobilinogen. Therefore, stools that appear pale may signify a blockage of the bile duct. Pale stools are also associated with diagnostic procedures that use barium sulfate.

A primary concern is the presence of blood in a stool specimen. Depending on the area of the intestinal tract from which bleeding occurs, the color can range from bright red to dark red to black. Blood that originates from the esophagus, stomach, or duodenum takes approximately 3 days to appear in the stool; during this time, degradation of hemoglobin produces the characteristic black, tarry stool. Likewise, blood from the lower gastrointestinal tract requires less time to appear and retains its original red color. Both black and red stools should be chemically tested for the presence of blood, because ingestion of iron, charcoal, or bismuth often produces a black stool, and medications and foods, including beets, produce a red stool.

Green stools may be observed in patients taking oral antibiotics, because of the oxidation of fecal bilirubin to biliverdin. Ingestion of increased amounts of green vegetables or food coloring also produces green stools.

Appearance

Besides variations in color, additional abnormalities that may be observed during the macroscopic examination include the watery consistency present in diarrhea and the small, hard stools seen with constipation. Slender, ribbon-like stools suggest an obstruction of the normal passage of material through the intestine.

Pale stools associated with biliary obstruction and steatorrhea appear bulky and frothy and frequently have a foul odor. Stools may appear greasy and may float.

The presence of mucus-coated stools is indicative of intestinal inflammation or irritation. Mucus-coated stools may be caused by pathologic colitis or excessive straining during elimination. Blood-streaked mucus suggests damage to the intestinal walls, possibly caused by bacterial or amebic *dysentery* or malignancy. The presence of mucus should be reported (Table 15–2).

Characteristics 12,26	
Color/Appearance	Possible Cause
Black	Upper gastrointestinal bleeding Iron therapy Charcoal
Red	Bismuth (antacids) Lower gastrointestinal bleeding Beets and food coloring Rifampin
Pale yellow, white, gray	Bile-duct obstruction Barium sulfate
Green	Biliverdin/oral antibiotics Green vegetables
Bulky/frothy	Bile-duct obstruction Pancreatic disorders
Ribbon-like	Intestinal constriction

Table 15-2 Macroscopic Stool

Microscopic Examination of Feces

Mucus/blood-streaked mucus Colitis

Microscopic screening of fecal smears is performed to detect the presence of leukocytes associated with microbial diarrhea and undigested muscle fibers and fats associated with steatorrhea.

Dysentery

Malignancy

Constipation

Fecal Leukocytes

Leukocytes, primarily neutrophils, are seen in the feces in conditions that affect the intestinal mucosa, such as ulcerative colitis and bacterial dysentery. Microscopic screening is performed as a preliminary test to determine whether diarrhea is being caused by invasive bacterial pathogens including *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, and enteroinvasive *E. coli*. Bacteria that cause diarrhea by toxin production, such as *Staphylococcus aureus* and *Vibrio* spp., viruses, and parasites usually do not cause the appearance of fecal leukocytes. Therefore, the presence or absence of fecal neutrophils can provide the physician with diagnostic information prior to the receiving of a culture report.

Specimens can be examined as wet preparations stained with methylene blue or as dried smears stained with Wright's or Gram stain. Methylene blue staining is the faster procedure but may be more difficult to interpret. Dried preparations

stained with either Wright's or Gram stains provide permanent slides for evaluation. An additional advantage of the Gram stain is the observation of gram-positive and gramnegative bacteria, which could aid in the initial treatment.³ All slide preparations must be performed on fresh specimens. In an examination of preparations under high power, as few as three neutrophils per high-power field can be indicative of an invasive condition.⁴ Using oil immersion, the finding of any neutrophils has approximately 70% sensitivity for the presence of invasive bacteria.⁵

A lactoferrin latex agglutination test is available for the detection of fecal leukocytes and remains sensitive in refrigerated and frozen specimens. The presence of lactoferrin, a component of granulocyte secondary granules, is indicative of an invasive bacterial pathogen.⁶

Muscle Fibers

Microscopic examination of the feces for the presence of undigested striated muscle fibers can be helpful in the diagnosis and monitoring of patients with *pancreatic insufficiency*, such as in cases of cystic fibrosis. It is frequently ordered in conjunction with microscopic examinations for fecal fats. Increased amounts of striated fibers may also be seen in biliary obstruction and *gastrocolic fistulas*.

Slides for muscle fiber detection are prepared by emulsifying a small amount of stool in 10% alcoholic eosin, which enhances the muscle fiber striations. The entire slide is examined for exactly 5 minutes, and the number of red-stained fibers with well-preserved striations is counted. Care must be taken to correctly classify the fibers observed. Undigested fibers have visible striations running both vertically and horizontally. Partially digested fibers exhibit striations in only one direction, and digested fibers have no visible striations. Only undigested fibers are counted, and the presence of more than 10 is reported as increased.

To produce a representative sample, patients should be instructed to include red meat in their diet prior to collecting the specimen. Specimens should be examined within 24 hours of collection.

Qualitative Fecal Fats

Specimens from suspected cases of steatorrhea can be screened microscopically for the presence of excess fecal fat.

PROCEDURE



Methylene Blue Stain Procedure for Fecal Leukocytes

- 1. Place mucus or a drop of liquid stool on a slide.
- 2. Add two drops Löffler methylene blue.
- 3. Mix with a wooden applicator stick.
- 4. Allow to stand 2–3 minutes.
- 5. Examine for neutrophils under high power.

PROCEDURE



Muscle Fiber Procedure

- 1. Emulsify a small amount of stool in two drops of 10% eosin in alcohol.
- 2. Coverslip and let stand 3 minutes.
- 3. Examine under high power for 5 minutes.
- 4. Count the number of undigested fibers.

The procedure can also be used to monitor patients undergoing treatment for malabsorption disorders. In general, correlation between the qualitative and quantitative fecal fat procedures is good; however, additional unstained phospholipids and cholesterol esters are measured by the quantitative procedure. Lipids included in the microscopic examination of feces are neutral fats (triglycerides), fatty acid salts (soaps), fatty acids, and cholesterol. Their presence can be observed microscopically by staining with the dyes Sudan III, Sudan IV, or oil red O; Sudan III is the most routinely used. The staining procedure consists of two parts, the neutral fat stain and the split fat stain.

Neutral fats are readily stained by Sudan III and appear as large orange-red droplets, often located near the edge of the coverslip. Observation of more than 60 droplets/highpower field can be indicative of steatorrhea; however, the split fat stain representing total fat content can provide a better indication. The breakdown of neutral fats by bacterial lipase and the spontaneous hydrolysis of neutral fats may lower the neutral fat count. This also precludes the comparison of the two slide tests to determine whether maldigestion or malabsorption is causing steatorrhea.

Soaps and fatty acids do not stain directly with Sudan III. Therefore, a second slide must be examined after the specimen has been mixed with acetic acid and heated. Examination of this slide reveals stained droplets that represent not only the free fatty acids but also the fatty acids produced by hydrolysis of the soaps and the neutral fats. In an examination of this split fat slide, both the number and size of the fat droplets must be considered. Normal specimens may contain

as many as 100 small droplets, less than 4 μ m in size, per high-power field. The same number of droplets measuring 1 to 8 μ m is considered slightly increased, and 100 droplets measuring 6 to 75 μ m is increased. ¹²

Cholesterol is stained by Sudan III after heating and as the specimen cools forms crystals that can be identified microscopically.

Chemical Testing of Feces

Occult Blood

By far the most frequently performed fecal analysis is the chemical screening test for the detection of occult (hidden) blood. As discussed previously, bleeding in the upper gastrointestinal tract may produce a black, tarry stool, and bleeding in the lower gastrointestinal tract may result in an overtly bloody stool. However, because any bleeding in excess of 2.5 mL/150 g of stool is considered pathologically significant, and no visible signs of bleeding may be present with this amount of blood, fecal occult blood testing (FOBT) is necessary. Originally used primarily to test suspected cases of gastrointestinal disease, FOBT has currently become widely used as a mass screening procedure for the early detection of colorectal cancer. Annual testing for occult blood has a high positive predictive value for detection of colorectal cancer in the early stages and is recommended by the American Cancer Society, particularly for persons older than age 50.13

The most frequently encountered screening tests for occult blood are based on detection of the pseudoperoxidase activity of hemoglobin. This is the same principle as the reagent strip test for urinary blood, but uses a different indicator chromogen. The reaction uses the pseudoperoxidase activity of hemoglobin reacting with hydrogen peroxide to oxidize a colorless compound to a colored compound:

$$\begin{array}{c} \text{Pseudo} \\ \text{Hemoglobin} \rightarrow \text{H}_2\text{O}_2 \rightarrow \text{Guaiac} \stackrel{0}{\rightarrow} \text{Oxidized guaiac} + \text{H}_2\text{O} \\ \text{Peroxidase} \end{array}$$

Several different indicator chromagens have been used to detect occult blood. All react in the same chemical manner but vary in their sensitivity. Listed in order of decreasing sen-

PROCEDURE



Neutral Fat Stain Procedure

- 1. Homogenize one part stool with two parts water.
- Mix emulsified stool with one drop 95% ethyl alcohol on slide.
- 3. Add two drops saturated Sudan III in 95% ethanol.
- 4. Mix and coverslip.
- 5. Examine under high power.
- 6. Count orange droplets per high-power field.

PROCEDURE



Split Fat Stain Procedure

- 1. Mix emulsified stool with one drop of 36% acetic
- 2. Add two drops saturated Sudan III.
- 3. Mix and coverslip.
- 4. Heat gently almost to boiling.
- **5.** Examine under high power.
- **6.** Count and measure the orange droplets per highpower field.

sitivity, these compounds include benzidine, ortho-tolidine, and gum guaiac. Contrary to most chemical testing, the least sensitive reagent, guaiac, is preferred for routine testing. Considering that a normal stool can contain up to 2.5 mL of blood, a less sensitive chemical reactant is understandably more desirable. In addition, pseudoperoxidase activity is present from hemoglobin and myoglobin in ingested meat and fish, certain vegetables and fruits, and some intestinal bacteria. Therefore, to prevent false-positive reactions, the sensitivity of the test must be decreased. This can be accomplished by varying the amount and purity of the guaiac reagent used in the test.

Many commercial testing kits are available for occult blood testing with guaiac reagent. The kits contain guaiac-impregnated filter paper, to which the fecal specimen and hydrogen peroxide are added. Two or three filter paper areas are provided for application of material taken from different areas of the stool, and positive and negative controls are also included. Obtaining samples from the center of the stool avoids false-positives from external contamination. Addition of hydrogen peroxide to the back of the filter paper that contains stool produces a blue color with guaiac reagent when pseudoperoxidase activity is present.

Packaging of the guaiac-impregnated filter paper in individually sealed containers has facilitated the screening program for colorectal cancer by allowing persons at home to place the specimen on the filter paper and bring or mail it to the laboratory for testing. To prevent false-positive reactions, specimens mailed to the laboratory should not be rehydrated prior to adding the hydrogen peroxide, unless specifically instructed by the kit manufacturer (Hemocult SENSA, Beckman Coulter, Fullerton, Calif.). Specimens applied to the paper in the laboratory should be allowed to dry prior to testing. The specimens should be tested within 6 days of collection. Two samples from three different stools should be tested before a negative result is confirmed. Patients should be instructed to avoid eating red meats, horseradish, melons, raw broccoli, cauliflower, radishes, and turnips for 3 days prior to specimen collection. This prevents the presence of dietary pseudoperoxidases in the stool. Aspirin and NSAIDs other than acetaminophen should not be taken for 7 days prior to specimen collection to prevent possible gastrointestinal irritation. Vitamin C and iron supplements containing vitamin C should be avoided for 3 days prior to collections, because ascorbic acid is a strong reducing agent that interferes with the peroxidase reaction.¹⁴

Additional, more sensitive and specific methods, for the detection of occult blood have been developed. Hemoquant (SmithKline Diagnostics, Sunnyvale, Calif.) provides a fluorometric test for hemoglobin and porphyrin. As hemoglobin progresses through the intestinal tract, bacterial actions degrade it to porphyrin that the guaiac test cannot detect. This can result in some false-negative results from upper gastrointestinal bleeding when using the guaiac test.

The immunochemical fecal occult blood test (iFOBT), Hemoccult ICT (Beckman Coulter, Fullerton, Calif.) is specific for the globin portion of human hemoglobin and uses anti-human hemoglobin antibodies. Because Hemoccult ICT is specific for human blood in feces, it does not require dietary or drug restrictions. It is more sensitive to lower GI bleeding that could be an indicator of colon cancer or other gastrointestinal disease and can be used for patients who are taking aspirin and other anti-inflammatory medications. The Hemoccult ICT does not detect bleeding from other sources such as a bleeding ulcer; thus decreasing the chance for false positives. Hemoglobin from upper GI bleeding is degraded by bacterial and digestive enzymes before reaching the large intestine and is immunochemically nonreactive. In contrast, there is little hemoglobin degradation in lower GI bleeding; therefore, the blood is immunochemically active. ¹⁵ Collection kits are similar to those used for guaiac testing and can be provided to patients for home collection.

Quantitative Fecal Fat Testing

Quantitative fecal fat analysis is used as a confirmatory test for steatorrhea. As discussed, quantitative fecal analysis requires the collection of at least a 3-day specimen. The patient must also maintain a regulated intake of fat (100 g/d) prior to and during the collection period. Paint cans make excellent collection containers because the specimen must be homogenized prior to analysis, and this can be accomplished by placing the container on a conventional paint-can shaker. Refrigerating the specimen prevents any bacterial degradation. The method routinely used for fecal fat measurement is the Van de Kamer titration, although gravimetric methods are available. Fecal lipids are converted to fatty acids and titrated to a neutral endpoint with sodium hydroxide. The fat

Summary of Occult Blood Testing Interference

False-Positive

Aspirin and anti-inflammatory medications

Red meat

Horseradish

Raw broccoli, cauliflower, radishes, turnips

Melons

Menstrual and hemorrhoid contamination

False-Negative

Vitamin C >250 mg/d

Iron supplements containing vitamin C

252 CHAPTER 15 • Fecal Analysis

content is reported as grams of fat or the coefficient of fat retention per 24 hours. Normal values based on a 100 g/d intake are 1 to 6 g/d or a coefficient of fat retention of at least 95%. The coefficient of fat retention is calculated as follows:

$$\frac{\text{(dietary fat - fecal fat)}}{\text{(dietary fat)}} \times 100$$

Although the Van de Kamer titration is the gold standard for fecal fat, the acid steatocrit is a rapid test to estimate the amount of fat excretion. It is similar to the microhematocrit test and is more convenient than a 72-hour stool collection. The acid steatocrit is a reliable tool to monitor a patient's response to therapy and screen for steatorrhea in pediatric populations. ^{16,17}

PROCEDURE



Acid Steatocrit Procedure

- 1. 0.5 g of feces from a spot collection is diluted 1 to 4 with deioinized water.
- 2. Vortex for 2 minutes to homogenize the specimen.
- 3. A volume of 5 N percholoric acid equal to 20% of the homogenate volume is added and the mixture is then vortexed for 30 seconds. Confirm the pH to be <1.
- **4.** Place the acid-homogenate mixture in 75 microliter plain hematocrit capillary tube. Seal the end with wax.
- 5. The capillary tube is centrifuged horizontally at 13,000 rpm for 15 minutes in a microhematocrit centrifuge. This separates fat as an upper layer overlying a solid fecal layer.
- The length of the fat and solid layers are measured using a magnifying lens.
- 7. Calculate the acid steatocrit in percent.
- 8. Calculate the fecal fat in grams per 24 hours.

 The acid steatocrit in percent = (fatty layer length in cm)/[(fatty layer length in cm) + (solid layer length)] × 100

The fecal fat for adults is quantitated as follows: Fecal fat in grams per 24 hours = $[0.45 \times (acid steatocrit in percent as a whole number)] - 0.43$

An acid steatocrit value <31% was considered normal while a value >31% indicated steatorrhea in adults.

The fecal fat for children up to the age of 15 years is as follows:

Fecal fat in grams per 24 hours =

 $[0.1939 \times (acid steatocrit in percent as a whole number)] - 0.2174$

Acid steatocrit is higher in infants and droppped with age. ¹⁸ An acid steatocrit of <10% is indicative of steatorrhea in children. ¹⁷

Near-infrared reflectance spectroscopy (NIRA) is a rapid procedure for fecal fat that requires less stool handling by laboratory personnel. The test requires a 48-to-72-hour stool collection to exclude day-to-day variability, but it does not require reagents after homogenization of the sample. The result is based on the measurement and computed processing of signal data from reflectance of fecal surface, which is scanned with infrared light between 1400 nM and 2600 nM wavelength. The results are calculated from calibration derived from known samples. The technique quantitates water, fat, and nitrogen in grams per 24 hours. ¹⁹ A summary of tests and current instrumentation for fecal fat analysis is presented in Table 15–3.

APT Test (Fetal Hemoglobin)

Grossly bloody stools and vomitus are sometimes seen in neonates as the result of swallowing maternal blood during delivery. Should it be necessary to distinguish between the presence of fetal blood or maternal blood in an infant's stool or vomitus, the APT test may be requested.

The material to be tested is emulsified in water to release hemoglobin (Hb) and, after centrifugation, 1% sodium hydroxide is added to the pink hemoglobin-containing supernatant. In the presence of alkali-resistant fetal hemoglobin, the solution remains pink (Hb F), whereas denaturation of the maternal hemoglobin (Hb A) produces a yellow-brown supernatant after standing for 2 minutes. The APT test distinguishes not only between fetal hemoglobin and hemoglobin A but also between maternal hemoglobins AS, CS, and SS, and fetal hemoglobin. The presence of maternal thalassemia major would produce erroneous results owing to

Table 15–3	Tests, Materials, and
	Instrumentation for
100	Fecal Fat Analysis 19

Procedure	Materials, Instrumentation
Sudan III	Sudan stain, microscopy
Steatocrit and acid steatocrit	Hematocrit centrifuge, gravi- metric assay
Fecal elastase–I	Immunoassay ELISA technique
Near-infrared reflectance spectroscopy (NIRA)	NIRA spectrophotometer. Wavelengths Range 1400–2600 nM. Computer Software for processing spectra
Van de Kamer	Fecal fat extraction and titration of long chain fatty acid by sodium hydroxide

PROCEDURE



APT Test Procedure

- 1. Emulsify specimen in water.
- 2. Centrifuge.
- 3. Divide pink supernatant into two tubes.
- 4. Add 1% sodium hydroxide to one tube.
- 5. Wait 2 minutes.
- 6. Compare color with that in the control tube.
- 7. Prepare controls using cord blood and adult blood.

the high concentration of hemoglobin F. Stool specimens should be tested when fresh. They may appear bloody but should not be black and tarry, because this would indicate already denatured hemoglobin.²⁰

Fecal Enzymes

Enzymes supplied to the gastrointestinal tract by the pancreas are essential for the digestion of dietary proteins, carbohydrates, and fats. A decrease in production of these enzymes (pancreatic insufficiency) is associated with disorders such as chronic pancreatitis and cystic fibrosis. Steatorrhea occurs, and there is the presence of undigested food in the feces.

Analysis of the feces focuses primarily on the proteolytic enzymes trypsin, chymotrypsin, and elastase I. Historically, absence of trypsin has been screened for by exposing x-ray paper to stool emulsified in water. When trypsin is present in the stool, it digests the gelatin on the paper, leaving a clear area. Inability to digest the gelatin indicates a deficiency in trypsin production. The gelatin test is an insensitive procedure that detects only severe cases of pancreatic insufficiency. In addition, false-negative results may occur as the result of intestinal degradation of trypsin and the possible presence of trypsin inhibitors in the feces. The proteolytic activity of bacteria enzymes may produce false-positive results in old specimens.

Fecal chymotrypsin is more resistant to intestinal degradation and is a more sensitive indicator of less severe cases of pancreatic insufficiency. It also remains stable in fecal specimens for up to 10 days at room temperature. Chymotrypsin is capable of gelatin hydrolysis but is most frequently measured by spectrophotometric methods.

Elastase I is an isoenzyme of the enzyme elastase and is the enzyme form produced by the pancreas. It is present in high concentrations in pancreatic secretions and is strongly resistant to degradation. It accounts for about 6% of all secreted pancreated enzymes. ²¹ Fecal elastase I is pancreas specific and its concentration is about five times higher than in pancreatic juice. It is not affected by motility disorders or mucosal defects. ²² Elastase I can be measured by immunoassay using the ELISA kit and provides a very

sensitive indicator of exocrine pancreatic insufficiency.^{23,24} It is easy to perform and requires only a single stool sample. The ELISA test uses monoclonal antibodies against human pancreatic elastase-1; therefore, the result is specific for human enzyme and not affected by pancreatic enzyme replacement therapy.²¹ The test is specific in differentiating pancreatic from nonpancreatic causes in patients with steatorrhea.²²

Carbohydrates

The presence of increased carbohydrates in the stool produces an osmotic diarrhea by the osmotic pressure of the unabsorbed sugar in the intestine drawing in fluid and electrolytes. Carbohydrates in the feces may be present as a result of intestinal inability to reabsorb carbohydrates, as is seen in celiac disease, or caused by lack of digestive enzymes such as lactase resulting in lactose intolerance. Idiopathic lactase deficiency is common, predominantly occurring in the African, Asian, and Southern European Greek populations. Carbohydrate malabsorption or intolerance (maldigestion) is primarily analyzed by serum and urine tests; however, an increased concentration of carbohydrate can be detected by performing a copper reduction test on the fecal specimen. Testing for fecal reducing substances detects congenital disaccharidase deficiencies as well as enzyme deficiencies due to nonspecific mucosal injury. Fecal carbohydrate testing is most valuable in assessing cases of infant diarrhea and may be accompanied by a pH determination. Normal stool pH is between 7 and 8; however, increased use of carbohydrates by intestinal bacterial fermentation increases the lactic acid level and lowers the pH to below 5.5 in cases of carbohydrate disorders.

The copper reduction test is performed using a Clinitest tablet (Siemens Medical Solutions Diagnostics, Tarrytown, N.Y.) and one part stool emulsified in two parts water. A result of 0.5 g/dL is considered indicative of carbohydrate intolerance. The Clinitest on stools can distinguish between diarrhea caused by abnormal excretion of reducing sugars and those caused by various viruses and parasites. Sucrose is not detected by the Clinitest method because it is not a reducing sugar. In premature infants there is correlation between a positive Clinitest and inflammatory necrotizing entercolitis. As discussed in Chapter 5, this is a general test for the presence of reducing substances, and a positive result would be followed by more specific serum carbohydrate tolerance tests, the most common being the D-xylose test for malabsorption and the lactose tolerance test for maldigestion. Stool chromatography to identify the malabsorbed carbohydrate is available but rarely necessary for the diagnosis of sugar intolerance. Small-bowel biopsy specimens for histologic examination and the assay of disaccharidase enzyme activity differentiate primary from secondary disaccharidase intolerance.²⁵

A summary of fecal screening tests is presented in Table 15-4.

Table 15-4 Summary of Fecal Screening Tests		
Test	Methodology/Principle	Interpretation
Examination for neutrophils	Microscopic count of neutrophils in smear stained with methylene blue, Gram stain, or Wright's stain	Three per high-power field indicates condition affecting intestinal wall
Qualitative fecal fats	Microscopic examination of direct smear stained with Sudan III	60 large orange-red droplets indicates malabsorption
	Microscopic examination of smear heated with acetic acid and Sudan III	100 orange-red droplets measuring 6–75 μm indicates malabsorption
Occult blood	Pseudoperoxidase activity of hemoglobin liberates oxygen from hydrogen peroxide to oxidize guaiac reagent	Blue color indicates gastrointestinal bleeding
APT test	Addition of sodium hydroxide to hemoglobin- containing emulsion determines presence of maternal or fetal blood	Pink color indicates presence of fetal blood
Trypsin	Emulsified specimen placed on x-ray paper determines ability to digest gelatin	Inability to digest gelatin indicates lack of trypsin
Clinitest	Addition of Clinitest tablet to emulsified stool detects presence of reducing substances	Reaction of 0.5 g/dL reducing substances suggests carbohydrate intolerance

References

- Singh, A, Gull, H, and Singh, R: Clinical significance of rapid (accelerated) gastric emptying. Clin Nucl Med 28(8):652-658, 2003.
- 2. Ukleja, A: Dumping syndrome: Pathophysiology and treatment. Nutr Clin Pract 20(5):517-525, 2005.
- 3. Koepke, JA: Tips from the clinical experts. MLO, p. 15, 1995.
- 4. Bradley, GM: Fecal analysis: Much more than an unpleasant necessity. Diagn Med 3(2):64-75, 1980.
- 5. Novak, R, et al: How useful are fecal neutrophil determinations? Lab Med 26(11):433, 1995.
- 6. McCray, WH, and Krevsky, B: Diagnosing diarrhea in adults: A practical approach. Hosp Med 34(4):27-36, 1998.
- 7. Walters, MP, et al: Clinical monitoring of steatorrhea in cystic fibrosis. Arch Dis Child 65:99-102, 1990.
- 8. Khouri, MR, Huang, G, and Shiau, YF: Sudan stain of fecal fat: New insight into an old test. Gastroenterology 96(2 Pt 1): 421-427, 1990.
- 9. Simko, V: Fecal fat microscopy. Am J Gastroenterol 75(3): 204-208, 1981.
- 10. Van de Kamer, JH, et al: A rapid method for determination of fat in feces. J Biol Chem 177:347-355, 1949.
- 11. Freeman, JA, and Beeler, MF: Laboratory Medicine: Urinalysis and Medical Microscopy. Lea & Febiger, Philadelphia, 1983.
- Drummey, GD, Benson, JA, and Jones, CM: Microscopic examination of the stool for steatorrhea. N Engl J Med 264:85-87, 1961
- 13. Mandel, JS, et al: The effect of fecal occult-blood screening on the incidence of colorectal cancer. N Engl J Med 343(22): 1603-1607, 2000.
- 14. Knight, KK, Fielding, JE, and Battista, RN: Occult blood screening for colorectal cancer. JAMA 261:587-590, 1989.

- 15. Hemoccult®ICT immunochemical fecal occult blood test package insert. Beckman Coulter, Fullerton, Calif., May 2005.
- Bijoor, AR, Geetha, S, and Venkateash, T: Faecal fat content in healthy adults by the "acid steatocrit method." Indian J Clin Biochem 19(2):20-22, 2004.
- 17. Van den Neucker AM, et al. Acid steatocrit: a reliable screening tool for steatorrhoea. Acta Paediatr 90:873-875, 2001.
- 18. Guarino, A., et al.: Reference values of the steatocrit and its modifications in diarrheal diseases. J Pediatr Gastroenterol Nutr 14:268-274, 1992.
- Serrano, PL, Navarro, JLL, and Fernandez-Rodriguez, CM: Laboratory tests and equipment for diagnostic work-up for malabsorption syndrome. LABTECH 2004.
- 20. Croak, M: Haemoglobin in stools from neonates: Measurement by a modified APT test. Med Lab Sci 48(4):346-350, 1991.
- Elphick, DA, and Kapur, K: Comparing the urinary pancreolauryl ratio and faecal elastase-1 as indicators of pancreatic insufficiency in clinical practice. Pancreatology 5:196-200, 2005.
- 22. Symersky, T, et al: Faecal elastase-I: Helpful in analysing steat-orrhoea? Neth J Med 62(8):286-289, 2004.
- Phillips, IJ, et al: Faecal elastase I: A marker of exocrine pancreatic insufficiency in cystic fibrosis. Ann Clin Chem 36:739-742, 1999.
- 24. Thorne, D, and O'Brien, C: Diagnosing chronic pancreatitis. Advance 12(14):8-12, 2000.
- Robayo-Torres, CC, Quezada-Calvillo, R, and Nichols, BL: Disaccharide digestion: Clinical and molecular aspects. Clin Gastroenterol Hepatol 4(3):276-287, 2006.
- Amann, ST, Josephson, SA, and Toskes PP: Acid steatocrit: A simple rapid gravimetric method to determine steatorrhea. Am J Gastroenterol 92:2280-2284, 1997.

STUDY UESTIONS

- 1. In what part of the digestive tract do pancreatic enzymes and bile salts contribute to digestion?
 - A. Large intestine
 - B. Liver
 - C. Small intestine
 - D. Stomach
- **2.** Where does the reabsorption of water take place in the primary digestive process?
 - A. Large intestine
 - B. Pancreas
 - C. Small intestine
 - D. Stomach
- **3.** Which of the following tests is *not* performed to detect osmotic diarrhea?
 - A. Clinitest
 - B. Fecal fats
 - C. Fecal neutrophils
 - D. Muscle fibers
- **4.** The normal composition of feces includes all of the following *except*:
 - A. Bacteria
 - B. Blood
 - C. Electrolytes
 - D. Water
- **5.** What is the fecal test that requires a 3-day specimen?
 - A. Fecal occult blood
 - B. APT test
 - C. Elastase I
 - D. Quantitative fecal fat testing
- **6**. The normal brown color of the feces is produced by:
 - A. Cellulose
 - B. Pancreatic enzyme
 - C. Undigested foodstuffs
 - D. Urobilin
- 7. Diarrhea can result from all of the following except:
 - A. Addition of pathogenic organisms to the normal intestinal flora
 - B. Disruption of the normal intestinal bacterial flora
 - C. Increased concentration of fecal electrolytes
 - D. Increased reabsorption of intestinal water and electrolytes
- **8.** Stools from persons with steatorrhea will contain excess amounts of:
 - A. Barium sulfate
 - B. Blood
 - C. Fat
 - D. Mucus

- **9.** Which of the following pairings of stool appearance and cause does *not* match?
 - A. Black, tarry: blood
 - B. Pale, frothy: steatorrhea
 - C. Yellow-gray: bile duct obstruction
 - D. Yellow-green: barium sulfate
- **10**. Stool specimens that appear ribbon-like are indicative of which condition?
 - A. Bile-duct obstruction
 - B. Colitis
 - C. Intestinal constriction
 - D. Malignancy
- 11. A black tarry stool is indicative of:
 - A. Upper GI bleeding
 - B. Lower GI bleeding
 - C. Excess fat
 - D. Excess carbohydrates
- **12**. Chemical screening tests performed on feces include all of the following *except*:
 - A. APT test
 - B. Clinitest
 - C. Pilocarpine iontophoresis
 - D. Trypsin digestion
- **13**. Secretory diarrhea is caused by:
 - A. Antibiotic administration
 - B. Lactose intolerance
 - C. Celiac sprue
 - D. Vibrio cholerae
- 14. The fecal osmotic gap is elevated in which disorder?
 - A. Dumping syndrome
 - B. Osmotic diarrhea
 - C. Secretory diarrhea
 - D. Steatorrhea
- **15.** Microscopic examination of stools provides preliminary information as to the cause of diarrhea because:
 - A. Neutrophils are present in conditions caused by toxin-producing bacteria
 - B. Neutrophils are present in conditions that affect the intestinal wall
 - C. Red and white blood cells are present if the cause is bacterial
 - D. Neutrophils are present if the condition is of non-bacterial etiology
- **16**. *True or False*: The presence of fecal neutrophils would be expected with diarrhea caused by a rotavirus.
- 17. Large orange-red droplets seen on direct microscopic examination of stools mixed with Sudan III represent:
 - A. Cholesterol
 - B. Fatty acids
 - C. Neutral fats
 - D. Soaps

Continued

- **18.** Microscopic examination of stools mixed with Sudan III and glacial acetic acid and then heated will show small orange-red droplets that represent:
 - A. Fatty acids and soaps
 - B. Fatty acids and neutral fats
 - C. Fatty acids, soaps, and neutral fats
 - D. Soaps
- **19.** When performing a microscopic stool examination for muscle fibers, the structures that should be counted:
 - A. Are coiled and stain blue
 - B. Contain no visible striations
 - C. Have two-dimensional striations
 - D. Have vertical striations and stain red
- 20. A value of 85% fat retention would indicate:
 - A. Dumping syndrome
 - B. Osmotic diarrhea
 - C. Secretory diarrhea
 - D. Steatorrhea
- **21**. Which of the following tests would *not* be indicative of steatorrhea?
 - A. Fecal elastase-I
 - B. Fecal occult blood
 - C. Sudan III
 - D. Van de Kamer
- **22.** Gum guaiac is preferred over ortho-tolidine for "occult" blood in mass screening tests because:
 - A. There is less interference from dietary hemoglobin
 - B. Ortho-tolidine is less sensitive
 - C. Gum guaiac reacts equally with formed and watery stools
 - D. Filter paper is more easily impregnated with gum guaiac
- 23. The term "occult" blood describes blood that:
 - A. Is produced in the lower GI tract
 - B. Is produced in the upper GI tract
 - C. Is not visibly apparent in the stool specimen
 - D. Produces a black, tarry stool
- **24.** What is the recommended number of samples that should be tested to confirm a negative occult blood result?
 - A. One random specimen
 - B. Two samples taken from different parts of three stools
 - C. Three samples taken from the outermost portion of the stool
 - D. Three samples taken from different parts of two stools

- **25.** Which test is more sensitive to upper GI bleeding?
 - A. Guaic fecal occult blood
 - B. Hemoquant
 - C. Immunochemical fecal occult blood
 - D. Sudan III
- **26**. Annual testing for fecal occult blood has a high predictive value for the detection of:
 - A. Colorectal cancer
 - B. Malabsorption syndromes
 - C. Pancreatic deficiencies
 - D. Ulcers
- **27**. Tests for the detection of "occult" blood rely on the:
 - A. Reaction of hemoglobin with hydrogen peroxide
 - B. Pseudoperoxidase activity of hemoglobin
 - C. Reaction of hemoglobin with ortho-tolidine
 - D. Pseudoperoxidase activity of hydrogen peroxide
- **28**. What is the significance of an APT test that remains pink after addition of sodium hydroxide?
 - A. Fecal fat is present.
 - B. Fetal hemoglobin is present.
 - C. Fecal trypsin is present.
 - D. Vitamin C is present.
- **29**. In the Van de Kamer method for quantitative fecal fat determinations, fecal lipids are:
 - A. Converted to fatty acids prior to titrating with sodium hydroxide
 - B. Homogenized and titrated to a neutral endpoint with sodium hydroxide
 - C. Measured gravimetrically after washing
 - D. Measured by spectrophotometer after addition of Sudan III
- **30.** A patient whose stool exhibits increased fats, undigested muscle fibers, and the inability to digest gelatin may have:
 - A. Bacterial dysentery
 - B. A duodenal ulcer
 - C. Cystic fibrosis
 - D. Lactose intolerance
- **31.** A stool specimen collected from an infant with diarrhea has a pH of 5.0. This result correlates with a:
 - A. Positive APT test
 - B. Negative trypsin test
 - C. Positive Clinitest
 - D. Negative occult blood test
- **32**. Which of the following tests differentiates a malabsorption cause from a maldigestion cause in steatorrhea?
 - A. APT test
 - B. D-xylose test
 - C. Lactose tolerance test
 - D. Occult blood test

Case Studies and Clinical Situations

- 1. Microscopic screening of a stool from a patient exhibiting prolonged diarrhea shows increased fecal neutrophils and normal qualitative fecal fats and meat fibers.
 - a. What type of diarrhea do these results suggest?
 - b. Name an additional test that could provide more diagnostic information.
 - c. Name one probable result for this test and one improbable result.
 - d. If the test for fecal neutrophils was negative and the fecal fat concentration increased, what type of diarrhea is suggested?
- 2. Laboratory studies are being performed on a 5-year-old boy to determine whether there is a metabolic reason for his continued failure to gain weight. In addition to having blood drawn, the patient has a sweat chloride collected, provides a random stool sample, and is asked to collect a 72-hour stool sample.
 - a. How can the presence of steatorrhea be screened for by testing the random stool sample?
 - b. How does this test distinguish among neutral fats, soaps, and fatty acids?
 - c. What confirmatory test should be performed?
 - d. Describe the appearance of the stool specimens if steatorrhea is present.
 - e. If a diagnosis of cystic fibrosis is suspected, state two screening tests that could be performed on a stool specimen to aid in the diagnosis.
 - f. State a possible reason for a false-negative reaction in each of these tests.
 - g. What confirmatory test could be performed?

- 3. A physician's office laboratory is experiencing inconsistencies in the results of patient-collected specimens for FOBT. Patients are instructed to submit samples from two areas of three different stools. Positive and negative controls are producing satisfactory results. Patient Number One is a 30-year-old woman taking over-thecounter medications for gastric reflux who has reported passing frequent black stools. The results of all three specimens are negative for occult blood. Patient Number Two is a 70-year-old woman suffering from arthritis. She is taking the test as part of a routine physical. The results of all three specimens are positive for occult blood. Patient Number Three is a 50-year-old man advised by the doctor to lose 30 lb. He has been doing well on a high-protein, low-carbohydrate diet. Two of his three specimens are positive for occult blood.
 - a. What is the possible nonpathologic cause of the unexpected results for Patient Number One? Patient Number Two? Patient Number Three?
 - b. How could the physician's office staff avoid these discrepancies?
 - c. What testing methodology could be used for Patients Number Two and Number Three?
- **4.** A watery black stool from a neonate is received in the laboratory with requests for an APT test, fecal pH, and a Clinitest.
 - a. Can all three tests be performed on this specimen? Why?
 - b. If the Clinitest is positive, what pH reading can be expected? Why?
 - c. The infant's hemoglobin remains constant at 18 g/dL. What was the significance of the black stool?
 - d. Would this infant be expected to have ketonuria? Why or why not?



Appendix A Urinalysis Automation

Studies have shown that the biggest variable in urinalysis testing is the conscientiousness of the laboratory personnel in their interpretations of the color reactions. This subjectivity associated with visual discrimination among colors has been alleviated by the development of automated reagent strip readers that use a spectrophotometric measurement of light reflection termed reflectance photometry. Reflectance photometry uses the principle that light reflection from the test pads decreases in proportion to the intensity of color produced by the concentration of the test substance. A monochromatic light source is directed toward the reagent pads by placing a filter between the light source and the reflective surface of the pad or by using a light-emitting diode (LED) to provide the specific wavelength needed for each test pad color reaction. The light is reflected to a photodetector and an analog/digital converter. The instruments compare the amount of light reflection with that of known concentrations, then display or print concentration units or transmit data to a laboratory information system (LIS). The ultimate goal of automation is to improve reproducibility and color discrimination while increasing productivity and standardization for reporting urinalysis results.

Several automated instruments are currently available to standardize sample processing, analyze test strips, perform urine sediment analysis, and report results with consistent quality and reduced hands-on time. Additional features include online computer capability with LIS interface; bar coding; manual entry of color, clarity, and microscopic results to be included on the printed report; flagging of abnormal results; storing of patient and control results; and minimal calibration, cleaning, and maintenance.

Automated instruments in urinalysis include individual strip readers, semiautomated analyzers, fully automated chemistry analyzers, automated urine cell analyzers, and completely automated systems. Semiautomated instruments are still dependent on an operator for specimen mixing, test strip dipping, and inputting of physical and microscopic results. Fully automated chemistry analyzers add urine to the reagent strip, and automated urine cell analyzers mix, aspirate, dilute, and stain urine to classify urine sediment particles. Automated urine systems perform a complete urinalysis that includes the physical, chemical, and microscopic parts of a routine urinalysis. The automated urinalysis instruments currently available are listed in Table A-1; however, new instruments are continually being developed.

The Clinitek 50 and Clinitek Status (Siemens Medical Solutions Diagnostics, Tarrytown, N.Y.) strip readers are well suited for small volume laboratories and physician's offices (Fig. A-1) and meet the Clinical Laboratory Improvement Amendments (CLIA) waived standards. Reagent strips

are manually dipped and placed on the strip reader, and results are displayed or printed. Patient identification and specimen color and clarity may be manually entered, abnormal results may be flagged, up to 100 test results for the Clinitek 50 and 200 tests results for the Clinitek Status may be stored in memory, and computer interfacing is available. An additional feature of these strip readers is their ability to provide an automated reading of microalbumin-to-creatinine and protein-to-creatinine ratios when using the Clinitek Microalbumin and Multistix PRO reagent strips and human chorionic gonadotropin (hCG) when the Clinitest hCG cassettes are used.

The Clinitek 200+ is designed for medium-volume to large-volume urinalysis laboratories and features a high specimen output of one strip every 10 seconds. Multistix reagent test strips are used, and the instrument has the ability to report semiquantitative (mg/dL) results or plus (+) and Système International (SI) units. All positive results are flagged to indicate a patient sample that requires additional confirmation testing or microscopic evaluation. The operator manually enters urine color and clarity observations and the patient identification number from a keyboard or an optional barcode reader. A bidirectional interface is available to upload and download patient identification information with the host computer. The reflectometer is calibrated daily and maintenance is required each day for all areas in contact with urine test strips.

The Clinitek 500 Urine Chemistry Analyzer (Siemens Medical Solutions Diagnostics, Tarrytown, N.Y.) (Fig. A-2) uses MULTISTIX 10 SG reagent strips for leukocytes, glucose, bilirubin, ketone, specific gravity, nitrite, pH, protein, urobilinogen, and blood. The Clinitek 500 utilizes a handheld bar-code reader that provides rapid entry of sample identification, color, and clarity values. This instrument includes automatic reagent strip detection, automatic calibration, confirmatory and microscopic sieve functions to flag results for quick review, and a user-friendly interface with a touch-screen display to provide a high-volume throughput of 500 strips per hour. Parameters for each analyte are set at installation to meet laboratory-specific protocol. Memory stores 500 patient results and 200 control results. Results are easily edited and reported by internal storing, transferring to the computer, or by printing. Calibration is automatic and maintenance is minimal.

The fully automated Clinitek Atlas (Siemens Medical Solutions Diagnostics, Tarrytown, N.Y.) is designed for a high-volume urinalysis laboratory and has the ability to load 50 samples (carousel) or 200 samples (rack) at one time with the capability to insert a stat specimen during the run (Fig. A-3). Regular urine sample tubes are used and can be placed in a

Div. Comany sign (acomation)		
Table A-1 Urinalysis Automation		
Equipment	Manufacturer	
Waived Urine Chemistry Instruments		
Clinitek 50	Siemens Medical Solutions Diagnostics	
Clinitek Status	Siemens Medical Solutions Diagnostics	
Chemstrip 101	Roche Diagnostics	
Ursys 1100 system	Roche Diagnostics	
Semiautomated Chemistry Instruments		
Clinitek 200/200+	Siemens Medical Solutions Diagnostics	
Clinitek 500	Siemens Medical Solutions Diagnostics	
Chemstrip Criterion II	Roche Diagnostics	
Chemstrip Urine Analyzer	Roche Diagnostics	
Urisys 1800 system	Roche Diagnostics	
Miditron Junior II	Roche Diagnostics	
Fully Automated Chemistry Instruments		
Clinitek Atlas	Siemens Medical Solutions Diagnostics	
Chemstrip Super Automated Urine Analyzer	Roche Diagnostics	
Urisys 2400 system	Roche Diagnostics	
Automated Microscopy		
UF-100 Urine Cell Analyzer	Sysmex	
iQ200 Automated Urine Microscopy	Iris Diagnostics Division	
Urine Analyzer (iQ200 Sprint)		
Automated Urinalysis Systems		
ADVIA Urinalysis WorkCell	Siemens Medical Solutions Diagnostics	
iQ200 Automated Urinalysis System	Iris Diagnostics Division	
CSF Analyzers		
ADVIA120	Siemens Medical Solutions Diagnostics	



ADVIA2120

Figure A–I Clinitek 50 Urine Chemistry Analyzer. (Courtesy of Bayer Healthcare Diagnostics, Tarrytown, N.Y.)

circular 50-position tray or in up to 20 (10-position) linear racks that are compatible with the Symex UF-100 Urine Cell Analyzer. Two milliliters of urine are required. STATS may be performed at any time. A reagent pack containing a roll of 490 dry reagent strips affixed to plastic support minimizes reagent handling. Tests are measured by the dry pad test using reflectance photometry to detect color change with readings taken at reactive and reference wavelength. Analytes measured are leukocytes, ketones, protein, glucose, nitrite, blood, urobilinogen, pH, bilirubin, color, creatinine, and protein-low. Specific gravity is measured by refractive index methodology, and clarity is a measurement of transmitted and scattered light. An exact volume of urine sample is pipetted onto the reagent test pad. Reagent pads advance automatically to the reflectance photometer to measure the color change of each reagent pad. Reagent pads then advance automatically to the disposal area. The Atlas uses bar-code sample identification

Siemens Medical Solutions Diagnostics



Figure A–2 Clinitek 500 Urine Chemistry Analyzer. (Courtesy of Bayer Healthcare Diagnostics, Tarrytown, N.Y.)

and allows abnormal ranges to be selected for identification and flagging of samples that require microscopic examination or confirmatory testing. One thousand patient results and 200 control results and calibrations are stored for visual display, printout, or transmission to a laboratory computer system. Standardized controls are run as set by laboratory protocol, and a 24-hour within-lot calibration is performed.

Roche Diagnostics Chemstrip 101 compact urine analyzer and the Roche Diagnostics Urisys 1100 system (Roche Diagnostics, Indianapolis, Ind.) provides simple test strip evaluation and also meets the standards for CLIA-waived testing. It is designed for point-of-care testing in small laboratories or physician office laboratories. Test strips are dipped and placed in a tray and a stepping motor moves it under the reading head. Incubation timing, analyte measurement by reflectance photometry, result calculation, and printout are automatic. Software options are available. One hundred patient samples are held in memory, and minimal calibration or maintenance is required. Roche Diagnostics instruments have urine compensation color pads and correct the results of the specific gravity test in strongly alkaline urine samples using Combur¹⁰ TestUX urine test strips (Roche Diagnostics, Indianapolis, Ind.).

The Chemstrip Criterion II (Roche Diagnostics, Indianapolis, Ind.), the Roche Diagnostics Urisys 1800 system, and the Roche Diagnostics Miditron Junior II are semiautomated urine test strip analyzers with upgraded software capa-



Figure A–3 Clinitek Atlas Automated Urine Chemistry Analyzer. (Courtesy of Bayer Healthcare Diagnostics, Tarrytown, N.Y.)

bility and are convenient and efficient for midsized laboratories. These instruments can test larger numbers of samples, including urine color, whereas clarity is entered manually. The strip is dipped into the sample and placed on the tray. Strip transport, test measurement, and disposal are automatic. Individual programming of result ranges, grading, and units is available. Instrument cleaning is minimal and is performed once a day; calibration is required twice a month. The Miditron Junior II (Roche Diagnostics, Indianapolis, Ind.) uses Combur¹⁰ Test M urine test strips (Roche Diagnostics) and can be set to perform 100 tests/hour (normal mode), 180 tests/hour (accelerated mode), or 300 tests/hour (fast mode) with a memory capacity of 150 results.

The Chemstrip Urine Analyzer (Roche Diagnostics, Indianapolis, Ind.), a semiautomated instrument, meets the needs of a large urinalysis laboratory by processing 300 strips per hour. It analyzes test strips placed on a transport tray, allows full sample identification, correlates and manages sediment microscopy data, and prints out or transmits results to the laboratory computer system. Bar coding enables microscopic sediment results to be entered and linked to test strip findings and patient data for correlation and patient assessment. Default settings are used to monitor the quality of results. Minimal maintenance is required because the instrument contains a cleaning cycle function and disposable transport and waste trays. Calibration is performed every 2 weeks and printed for a permanent report.

The Urisys 1800 reduces hands-on time by a continuous loading of test strips that use a sensor for automatic strip detection. With a Urisys 1800 sediment terminal, the test strip results and microscopic results are consolidated for reporting. Results and control and calibration data are stored on a floppy disk.

The Chemstrip Super Automated Urine Analyzer (Roche Diagnostics, Indianapolis, Ind.) and the Roche Diagnostics Urisys 2400 system are fully automated "walk-away" urine

chemistry instruments that meet the needs of a large urinalysis laboratory. With the Chemstrip Super Automated Urine Analyzer, urine specimens are loaded in a 60-position carousel with 55 routine positions and 5 stat positions. Sample volumes are detected, adjusted, and automatically mixed. A sorter mechanism supplies a single test strip from the sorter drum to a sorter position. A gripping mechanism grasps the test strip and dips it into the urine specimen tube. A sensor attached to a mixing rod determines the volume of urine. The dipping mechanism lifts the test strip out of the sample tube while removing excess urine by dragging the strip along the inside of the specimen tube. The dipping mechanism then transfers the test strip to the reflectance photometer position. A transport plate positions the test strip at the reflectance photometer recording head, where the specimen is measured at three different wavelengths (555, 620, 660 nm) at 48 seconds and/or 120 seconds after dipping. The result is converted to a concentration value and printed or transferred to a laboratory computer. An optional built-in bar-code reader is available for patient identification. The instrument is calibrated with a special calibration strip once every 2 weeks.

The Urisys 2400 system utilizes a pipetting unit that automatically mixes the specimen and pipettes the precise volume to each test pad. The minimum sample volume is 1.5 mL. Four hundred test strips are loaded into a Urisys 2400 cassette and the strips are stable in the cassette for 2 weeks. The instrument has the capability of measuring 75 samples per load with a dedicated STAT position for immediate measurement of emergency samples. Calibration is performed with a calibration strip only once a month.

The AUTION MAX AX-4280 automated urine chemistry analyzer (Iris Diagnostics, Chatsworth, Calif.) performs up to 210 patient specimens. It is a fully automated urine chemistry instrument that auto-identifies and processes bar-coded specimen tubes in 10-position racks by mixing, sampling, and analyzing the urine specimen. A 1.0 mL urine sample is aspirated and the correct amount is deposited on the reagent strip and the remainder of the sample is taken to the spectrophotometer and refractometer for color, clarity, and specific gravity measurements. Dual wave reflectance and a color compensation pad limit interferences from color, turbidity and drugs.

AUTOMATED MICROSCOPY In a routine urinalysis, a test strip determines the chemical analytes, and the formed elements are determined by microscopy. Manual microscopy is not easily standardized because of the high variation among operators even in the same institution. Intensive specimen processing affects accuracy as rare elements such as casts or crystals may be lost during handling. Results are not quantitative because they must be reported in ranges or averages. Overall, manual microscopy is not cost effective because of the poor use of personnel in-batch processing and poor turnaround time for STATs. Automated urine cell analyzers provide efficient standardized results in about a minute, markedly improving turnaround times.

The Sysmex UF-Series offers fully automated sample analysis with automatic classification of all 10 formed element

groups with scattergrams and histograms for reference. The Sysmex UF-100 Urine Cell Analyzer (Sysmex America, Inc., Mundelein, Ill.) (Fig. A-4) is designed for large urinalysis laboratories with predominantly normal microscopic results and can process 100 samples per hour; the UF-50 processes 50 samples per hour. The UF-100 and UF-50 use laser-based flow cytometry along with impedance detection, forward light scatter, and fluorescence to identify the individual characteristics and stained urine sediment particles in a flowing stream. The instrument is easy to operate by placing a 10-position linear rack on the instrument and initiating the autoanalysis with a touch of the screen. Uncentrifuged urine is aspirated into the instrument and the conductivity is measured. The sample is stained with two dyes that radiate an orange and green fluorescence. The DNA within the cells is stained by the orange dye, phenathridine; the nuclear membranes, mitochondria, and negatively charged cell membranes are stained with a green dye, carbocyanine. The stained sample is passed through the flow cell, where it is hydrodynamically focused and presented to a laser light beam (488 nm) that produces fluorescence and light scatter. Particles are identified by measuring the change in impedance of the sediment elements, as well as the height and width of the fluorescent and light scatter signals, which are presented in scattergrams and histograms. The width of the fluorescent signal measures cellular inclusions and the width of forward light scatter measures the length of cells. Values are presented in a numerical quantitation (cells per microliter) and cells per high- or low-powered field, using a standard conversion factor in the instrument software. Thresholds to be flagged for primary elements can be established and abnormal results are "flagged" for confirmatory review. The main parameters are red blood cells (RBCs), white blood cells (WBCs), epithelial cells, casts, and bacteria. Flagging parameters include pathologic casts, crystals, small round cells, sperm, and yeastlike cells. An internal quality control system monitors performance, and quality control records can be viewed on the instrument screen in a Levy-Jennings graph. One thousand patient results including scattergrams, histograms, and specimen characteristics are stored. A bidirectional interface is provided to download and report results. The UF-50 or UF-100 can be used independently or integrated with an automated urine chemistry strip reader to create a complete urinalysis system by choosing the UF autosampler that is compatible.

The iQ 200 Automated Urine Microscopy Analyzer (Iris Diagnostics, Chatsworth, Calif.) automatically analyzes and classifies urine particles into 12 categories. The sample is mixed and aspirated to a planar flowcell where 500 digital photomicroscopic images are taken per sample. The system uses Auto Particle Recognition (APR) software that classifies urine particles in the photographs based on size, shape, texture, and contrast into 12 categories—RBCs, WBCs, WBC clumps, hyaline casts, unclassified casts, squamous epithelial cells, nonsquamous epithelial cells, bacteria, yeast, crystals, mucus, and sperm. Particle identification is confirmed, or flagged and checked by a technologist before



Figure A-4 UF-100 Automated Urine Cell Analyzer. (Courtesy of Sysmex, Mundelein, III.)

released. Results are sent to an operator screen or directly to the LIS. Because the photographs are digitally archived, results can be reviewed easily.

AUTOMATED URINALYSIS SYSTEMS Turnaround times for a complete urinalysis have significantly improved by reducing the technologist's hands-on time with the addition of completely automated urinalysis systems by combining automated urine chemistry analyzers and automated urine cell analyzers. Using similar sample racks and moving on a conveyor system, samples are easily transferred from one instrument to the next, providing a complete walkaway capability with minimal specimen handling from sampling of the specimen to reporting test results. By interfacing with the LIS, results that are standardized and reliable are transmitted error-free.

The Clinitek Atlas (Siemens Medical Solutions Diagnostics, Tarrytown, N.Y.), an automated urine chemistry analyzer, and the Sysmex UF-100 (Sysmex America, Inc, Mundelein, Ill.), an automated urine cell analyzer, have been

integrated to develop the ADVIA Urinalysis Workcell System (Siemens Medical Solutions Diagnostics, Tarrytown, N.Y.). CEREBROSPINAL FLUID ASSAY Traditionally, cerebrospinal fluid (CSF) cell counts and differentials are performed manually using a Neubauer hemocytometer and are a labor-intensive and time-consuming procedure subject to technologist variability. Automation provides quality control and precision for a method that previously was uncontrolled. The ADVIA120 Hematology System (Siemens Medical Solutions Diagnostics, Tarrytown, N.Y.) was the first automated instrument with an FDA-approved automated CSF assay. The ADVIA2120 Hematology System has built upon the ADVIA120 technology and is also used for CSF assays. The instruments use flow cytometry, light scatter, and absorbance to count the RBCs, WBCs, and perform a WBC differential that includes percentages and absolute numbers of mononuclear cells and polymorphonuclear cells on samples with greater than 20 WBC/µL. The WBC differential includes the numbers of neutrophils, lymphocytes, monocytes, and eosinophils. The CSF specimen is pretreated with CSF reagent to fix and spherize the cells. The prepared sample is stable between 4 minutes and 4 hours when stored from 18°C to 30°C. The specimen is aspirated into the instrument and cells are differentiated and enumerated by three optical measurements. The signals are digitized and used to construct the CSF cytogram. With this system, more cells are counted, achieving increased accuracy and precision. The automated RBC, WBC, polymorphonuclear, mononuclear, and differential results are available within 1 minute of aspiration of the sample. In addition, the ADVIA 2120 can be used for counting lamellar bodies in amniotic fluid to determine fetal lung maturity. Lamellar bodies can be counted in the platelet channel by using high and low laser light scatter. This provides a rapid automated diagnostic test for fetal lung maturity.

ADDITIONAL INFORMATION SOURCES

Siemens Medical Solutions Diagnostics, Tarrytown, NY: www. medical.siemens.com

International Remote Imaging Systems: www. irisdiagnostics.com

Roche Diagnostics: www.rochediagnostics.com Sysmex America, Inc.: www.sysmex.com/usa



Appendix B Bronchoalveolar Lavage

The analysis of specimens obtained by bronchoalveolar lavage (BAL) provides a method for obtaining cellular and microbiological information from the lower respiratory tract. Saline infused by bronchoscope mixes with the bronchial contents and is aspirated for cellular examination and culture. BAL is particularly useful in evaluating immunocompromised patients, interstitial lung disease, airway diseases, and suspected alveolar hemorrhage.

BAL specimens are usually separated into two samples and designated as the bronchial sample and the alveolar sample. The bronchial sample is the first aliquot instilled and recovered. The alveolar sample consists of the subsequent 3 to 5 aliquots, which are instilled and recovered. These aliquots are usually combined. Specimens should be analyzed immediately. Cell counts must be performed within 1 hour.

In the hematology laboratory, a gross observation is recorded describing the color and clarity of the specimen. The presence of clots should be noted. The fluid volume is measured and cell counts and differential counts are performed. White blood cell (WBC) and red blood cell (RBC) counts are performed on BAL and may be diluted to facilitate counting using a hemocytometer.

WBC counts may be diluted using a Unopette diluting system. Either the 1/100 ammonium oxalate or the 1/20 glacial acetic acid Unopette systems are available to lyse the RBCs. When the RBCs have lysed and the solution is clear, the fluid is plated on a hemocytometer and the cells are allowed to settle for 5 minutes. All cells in the 18 squares are counted (both sides of the hemacytometer) and the average of the two sides is calculated. Using the following formula, the WBC count is calculated.¹

$$WBC/cmm = \frac{\text{factor} \times 10}{9 \text{ squares}}$$

RBC counts may be diluted with isotonic saline. The fluid is plated on a hemocytometer and allowed to settle for 5 minutes. Both sides of the hemocytometer are counted and the RBC/cmm is calculated using the following formula.¹

$$RBC/cmm = \frac{\text{number of cells} \times \text{dilution factor} \times 10}{\text{Number of squares counted}}$$

Differential slides are prepared by cytocentrifugation using routine procedures, and at least 300 cells but often 500 to 1000 cells are counted and classified.² Cells seen in BAL fluid include macrophages, lymphocytes, neutrophils, eosinophils, ciliated columnar bronchial epithelial cells, and squamous epithelial cells.

Macrophages, often containing a variety of phagocytized material, are the cells most frequently seen, with numbers ranging from 56% to 80% (Fig. B-1).

Lymphocytes, normally constituting 1% to 15% of the cell population, are increased in interstitial lung disease, drug reactions, pulmonary lymphoma, and nonbacterial infections. The ratio of CD4 to CD8 lymphocytes further defines the disease process. An elevated CD4/CD8 indicates *sarcoidosis* or connective tissue disorders. A normal CD4/CD8 is associated with tuberculosis or malignancies, whereas a low CD4/CD8 is indicative of hypersensitivity pneumonitis, silicosis, druginduced disease, or HIV infection.³

Neutrophils are the primary granulocyte seen, with a normal value of less than 3%. They are elevated in cigarette smokers, bronchopneumonia, toxin exposure, and diffuse alveolar damage.

Eosinophils, usually less than 1% to 2% of the total cells, are elevated in asthma, drug-induced lung disease, infections (parasitic, mycobacterial, or fungal), hypersensitivity, pneumonitis, and eosinophilic pneumonia.

Erythrocytes are an indication of an alveolar hemorrhage within the first several hours. Phagocytosed erythrocytes suggest an alveolar hemorrhage within 48 hours, whereas hemosiderin-laden macrophages indicate an alveolar hemorrhage greater than 48 hours.

Ciliated columnar bronchial epithelial cells are more numerous in bronchial wash specimens than bronchial lavage specimens because of the more vigorous washing technique. In a lavage specimen, they normally range from 4% to 17% (Fig. B-2).

Fungal elements and viral inclusions may also be observed in respiratory specimens. Organisms identified include Pneumoncystis carinii, Toxoplasma gondii, Strongyloides stercoralis, Legionella pneumophila, Cryptococcus neoformans, Histoplasma capsulatum, Mycobacterium tuberculosis, Mycoplasma pneumoniae, influenza A and B viruses, and respi-

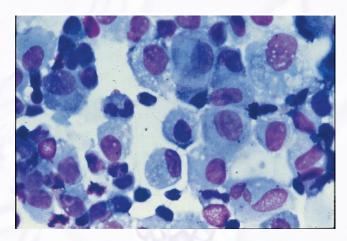


Figure B–I Bronchoalveolar lavage: Normal macrophages and lymphocytes (×1000).

ratory syncytial virus.3 Quantitative or semiquantitative cultures are useful for ventilator-associated pneumonia and are diagnostic of the infection if the organism is identified.³ With the increasing concern about nosocomial infections and antibiotic-resistant microorganisms, BAL is more frequently performed on ventilator-assisted patients to detect infection and for monitoring antibiotic therapy.

Bronchoalveolar lavage is becoming an important diagnostic test for Pneumocystis carinii in immunocompromised patients. With Pneumocystis carinii, characteristic amorphous material is seen microscopically under low power and organisms are visible under high power (Figs. B-3 and B-4).4

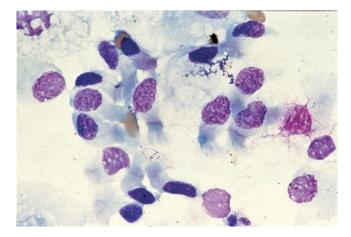


Figure B-2 Bronchoalveolar lavage: Ciliated bronchial epithelial cells; notice the eosinophilic bar ($\times 1000$).

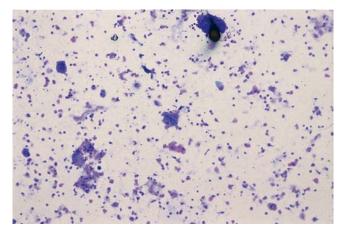


Figure B-3 Bronchoalveolar lavage: Amorphous material associated with Pneumocystis carinii when examined under low power (X100).

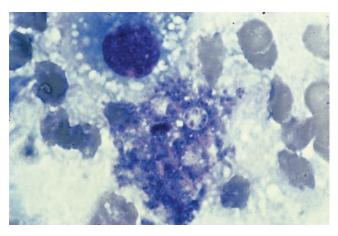


Figure B-4 Bronchoalveolar lavage: Characteristic cup-shaped organisms indicative of Pneumocystis carinii (×1000).

Cryptococcus neoformans has become a significant opportunistic pathogen in patients with AIDS. A diagnosis of pulmonary cryptococcosis can be made by demonstrating a positive cryptococcal antigen in respiratory specimens exhibiting yeast cells that morphologically resemble C. neoformans. The extent of the cryptococcal infection correlates with the antigen titer.5

Cytologic studies include observing sulfur granules (actinomycetes), hemosiderin-laden macrophages, Langerhans cells, cytomegalic cells, fat droplets seen in fat embolism with an Oil red O stain, and lipid-laden alveolar macrophages using a Sudan III stain. Dust particle inclusions are indicative of pneumoconioses or asbestos exposure.3

References

- 1. Methodist Hospital: Clinical Laboratory Procedure Manual. Omaha, Nebr., April 13, 2006.
- 2. Jacobs, JA, DeBrauwer, EI, et al: Accuracy and Precision of Quantitative Calibrated Loops in Transfer of Bronchoalveolar Lavage Fluid. J Clin Micro, 38(6):2117-2121, 2000.
- 3. American Thoracic Society: Bronchoalveolar Lavage. http://www.thoracic.org/sections/clinical-information/ critical-care/atlas-of-critical-care-procedures/procedures/ bronchoalveolar-lavage.cfm, May 2004. Accessed January 2007.
- 4. Linder, J. Bronchoalveolar Lavage. ASCP, Chicago, 1988.
- 5. Bottone, EJ, Sindone, M, Caraballi, V: Value of Assessing Cryptococcal Antigen in Bronchoalveolar Lavage and Sputum from Patients with AIDS. Presented in part at the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy, Orlando, Fla., May 1994. Abstract B8. http://www.mssm.edu/ msjournal/65/18_Bottone.pdf. Accessed January 22, 2007.



Answers to Case Studies and Clinical Situations

Chapter 2

- 1. a. 160 to 180 mg/dL
 - b. Renal tubular reabsorption is impaired.
- a. Juxtaglomerular apparatus →
 Macula densa → Juxtaglomerular apparatus →
 Angiotensinogen →Renin →Angiotensin I →
 Angiotensin II.
 - b. Vasodilation and constriction, increased sodium reabsorption, increased aldosterone and ADH production.
 - c. Production of renin decreases, and this reduces the actions of the renin-angiotensin-aldosterone system.
- 3. a. The physician can calculate the approximate creatinine clearance using the Cockcroft-Gault formula.
 - b. Yes, the calculated creatinine clearance is 80 mL/min, a normal value for a male of this age.
 - c. Yes, the measured creatinine clearance is 86 mL/min, normal for this patient.
- 4. a. Serum from the midnight specimen is not being separated from the clot and refrigerated in a timely manner
 - b. Yes, lactic acid affects both cryoscopic and vapor pressure osmolarity readings.
 - c. If the laboratory is using a cryoscopic osmometer, results will be affected by alcohol ingestion; vapor pressure results would not be affected and could be used as a comparison.
- 5. a. Diabetes insipidus.
 - b. Decreased production of ADH.
 - c. Lack of tubular response to ADH.

Chapter 3

- 1. a. No, the specimen should be analyzed and the volume reported. Persons with decreased glomerular filtration rates may also exhibit oliguria.
 - b. Failure to collect a complete specimen will result in a low clearance rate not related to renal function.
- 2. a. The specimen is a dilute random specimen that has remained more than 2 hours at room temperature prior to being tested.
 - b. Collect a 2-hour postprandial specimen.
- 3. a. A prostatic infection cannot be determined because the patient has a urinary tract infection.
 - b. White blood cells present after prostate massage only need to be compared with one premassage specimen.
 - c. A prostate infection is present.

- 4. a. The specimen temperature was measured.
 - b. The temperature was too low.
 - c. The specimen analyzed did not belong to the defendant
 - d. By maintaining a thoroughly documented COC form.

Chapter 4

- 1. a. No, because red blood cells would produce a hazy cloudy specimen.
 - b. Hemoglobin from intravascular hemolysis and myoglobin from muscle-tissue damage.
 - c. Yes, the red blood cells may have hemolyzed.
 - d. Dietary intake, exercise, and medications.
- 2. a. Yes, the specimen may contain melanin or homogentisic acid.
 - b. Homogentisic acid.
 - c. Melanin.
 - d. Breakdown of red blood cells followed by oxidation of hemoglobin to methemoglobin.
- 3. a. Radiographic dye.
 - b. 1.060
 - c. Add 3 mL of water to 1 mL of urine and mix.
 - d. Reagent strip
- 4. a. Beets
 - b. Yes, beets produce a red color in alkaline urine, and the fresh specimen may have an acid pH, or she has not recently eaten beets.
- 5. a. **Support:** the specimen may be old and bilirubin has oxidized to biliverdin and glycolysis may have occurred.
 - b. **Disagree:** The specimen is concentrated and the white foam is from protein that will be detected by reagent strip.
 - c. **Support**: The specimen may be old.
 - d. **Disagree:** The specimen can be accurately analyzed by reagent strip.

Chapter 5

- 1. a. The patient's blood glucose level exceeds the renal threshold for glucose, causing glucosuria.
 - b. Diabetes mellitus.
 - c. Diabetic nephropathy.
 - d. Periodic testing for microalbuminuria followed by better stabilization of the blood glucose levels.
 - e. Tubular dysfunction

Answers to Case Studies and Clinical Situations

- 2. a. Yellow foam
 - b. Ictotest
 - c. Possible biliary-duct obstruction preventing conjugated bilirubin from entering the intestine.
 - d. Icteric
 - e. Specimens must be protected from light.
- 3. a. Hemoglobinuria
 - b. Increased bilirubin presented to the liver results in increased bilirubin entering the intestine for conversion to urobilinogen.
 - c. The circulating bilirubin is unconjugated.
 - d. Perform a Watson-Schwartz test; retest the specimen using a Chemstrip.
- 4. a. Negative chemical reactions for blood and nitrite.

 Ascorbic acid interference for both reactions. Random specimen or further reduction of nitrite could cause the negative nitrite.
 - b. Glucose, bilirubin, LE. Ascorbic acid is a strong reducing agent that interfers with the oxidation reaction in the glucose test. Ascorbic acid combines with the diazo reagent in the bilirubina and LE tests lowering the sensitivity.
 - c. The dark yellow color may be caused by beta-carotene instead of specimen concentration.
 - d. Non-nitrate-reducing microorganisms; lack of dietary nitrate; antibiotic administration.
- 5. a. To check for possible exercise-induced abnormal results
 - b. Negative protein and blood, possible changes in color and specific gravity.
 - c. Renal
- 6. a. No, the specimen is clear.
 - b. Myoglobinuria
 - c. Muscle damage from the accident (rhabdomyolysis).
 - d. Yes, myoglobin is toxic to the renal tubules.
- 7. a. Laboratory personnel are not tightly capping the reagent strip containers in a timely manner.
 - b. Personnel performing the manual reagent strip test are not waiting 2 minutes to read the LE reaction.
 - c. The student is not mixing the specimen.

Chapter 6

- 1. a. Yeast grows best at a low pH with an increased concentration of glucose.
 - b. Yes, this exceeds the renal threshold.
 - c. No, yeast is not capable of reducing nitrate to nitrite.
 - d. Moderate blood with no RBCs.
 - e. Myoglobin is the cause of the positive chemical test result for blood. The patient has been bedridden for an extended period of time, causing muscle destruction.
- 2. a. The large objects are in a different plane than the urinary constituents.
 - b. Contamination by artifacts.
 - c. No, because they are in a different plane.
 - d. Polarizing microscopy.

- 3. a. Renal tubules.
 - b. Yes, viral infections can cause tubular damage.
 - c. RTE cells absorb the bilirubin-containing urinary fil-
 - d. Liver damage inhibits processing of reabsorbed urobilinogen.
 - e. Disorders producing intravascular hemolysis.
- 4. a. The patient is taking a pigmented medication, such as phenazopyridine.
 - b. Yes
 - c. An Ictotest could be run but is not indicated by the patient's symptoms.
 - d. Ask what medications the patient is taking.
 - e. Ampicillin
- 5. a. Calcium oxalate.
 - b. Monohydrate and dihydrate calcium oxalate.
 - c. Oval/dumbell: monohydrate; envelope: dihydrate.
 - d. Monohydrate.
- 6. a. Microscopic results do not match the chemical tests for blood, nitrite, and leukocyte esterase.
 - b. The specimen has been unpreserved at room temperature for too long, the cells have disintegrated, and the bacteria have converted the nitrite to nitrogen.
 - c. The pH.
 - d. Ask the clinic personnel to instruct the patient to collect a midstream clean-catch specimen and have the specimen delivered immediately to the laboratory.
- 7. a. No, because they are associated with strenuous exercise.
 - b. The positive blood reaction is from hemoglobinuria or myoglobinuria resulting from participating in a contact sport. The protein is orthostatic.
 - c. Increased excretion of RTE cell lysosomes in the presence of dehydration.
- 8. a. Yes, the waxy casts are probably an artifact, such as a diaper fiber. Waxy casts are not associated with negative urine protein.
 - b. No, this is normal following an invasive procedure.
 - c. Yes, tyrosine crystals are seen in severe liver disease; therefore, the bilirubin should be positive. The crystals may be an artifact or from a medication.
 - d. Yes, uric acid crystals may be mistaken for cystine crystals.
 - e. Yes, radiographic dye crystals associated with a high specific gravity resemble cholesterol crystals.
 - f. No, Trichomonas is carried asymptomatically by men.
 - g. Yes, calcium carbonate crystals are found in alkaline urine; therefore, clumps of amorphous urates may be mistaken for calcium carbonate crystals.

Chapter 7

- 1. a. Review of the procedure by a designated authority has not been documented.
 - b. Instructions and training are not being provided to personnel performing collections.
 - c. A safety statement about the heat produced by the reaction is not in the procedure manual.
 - d. The bottles have not been dated and initialed.

- a. Yes, provided you comply with the proficiency testing, patient test management, and QC and QA requirements for moderate complexity testing.
 - b. The CLIA status will be moderate complexity, proficiency testing will be required, and inspections will be conducted.
- 3. a. The PDCA strategy.
 - b. Analyze the problems, using flowcharts, cause-and-effect, and/or Pareto charts, and discuss theories to correct the problems. Test the recommended changes, analyze the results of these tests, and implement effective changes.
- a. Correct; proficiency survey tests should be rotated among personnel performing the tests.
 - b. Accept; QC on the Clinitest tablets must only be performed when they are used to perform a test.
 - c. Correct; documentation of technical competency should be performed on all personnel working in the section and educational qualifications assessed.

Chapter 8

- 1. a. Acute glomerulonephritis.
 - b. M protein in the cell wall of the group A streptococcus.
 - c. Glomerular bleeding.
 - d. No, they are also passing through the damaged glomerulus.
 - e. Good prognosis with appropriate management of secondary complications.
 - f. Henoch-Schönlein purpura.
- 2. a. IgA nephropathy/Berger disease
 - b. Serum IgA level
 - c. Chronic glomerulonephritis/end-stage renal disease.
 - d. Impaired renal tubular function associated with end-stage renal disease.
 - e. The specific gravity is the same as that of the ultrafiltrate, indicating a lack of tubular concentration.
 - f. The presence of extreme urinary stasis.
- 3. a. Nephrotic syndrome.
 - b. Nephrotic syndrome may be caused by sudden, severe hypotension.
 - c. Changes in the electrical charges in the glomerular membrane produce increased membrane permeability.
 - d. Decreased plasma albumin lowers the capillary oncotic pressure causing fluid to enter the interstitial tissue.
 - e. Reabsorption of filtered lipids by the RTE cells.
 - f. Staining with Sudan III and observation under polarized light.
- 4. a. Minimal change disease.
 - b. Nephrotic syndrome, focal segmental glomerulosclerosis
 - c. Good prognosis with complete remission.
- 5. a. Goodpasture syndrome.
 - b. The autoantibody attaches to the glomerular capillaries, causing complement activation and destruction of the capillaries.

- c. Wegener granulomatosis.
- d. Antineutrophilic cytoplasmic antibody.
- e. Granuloma formation resulting from autoantibodies binding to neutrophils in the vascular walls and initiating an immune response.
- 6. a. Cystitis, UTI.
 - b. The specimen is very dilute.
 - c. Irritation of the urinary tract will cause a small amount of bleeding. The cells and bacteria may cause a trace protein, or it may be a false positive due to the high pH.
 - d. Yes, glitter cells are seen in hypotonic urine.
 - e. Female children.
 - f. Pyelonephritis.
- 7. a. Intravenous pyelogram.
 - b. Chronic pyelonephritis.
 - c. WBC cast.
 - d. Reflux nephropathy.
 - e. Performing a Gram stain.
 - f. Radiographic dye.
 - g. Permanent tubular damage and progression to chronic, end-stage renal disease.
- 8. a. Abnormal
 - b. Acute interstitial nephritis.
 - c. This disorder is an inflammation not an infection.
 - d. Discontinue the medication because it is causing the allergic reaction.
- 9. a. Acute renal failure.
 - b. The prerenal sudden decrease in blood flow to the kidneys.
 - c. Lack of renal concentrating ability.
 - d. Tubular damage.
 - e. The increased diameter of the damaged distal convoluted tubule and extreme urinary stasis allowing casts to form in the collecting ducts.
- 10. a. Renal lithiasis.
 - b. The high specific gravity.
 - c. Yes, the dark yellow color and high specific gravity indicate a concentrated urine, which induces the formation of renal calculi.
 - d. Calcium oxalate.
 - e. Increased hydration and possible dietary changes.
- 11. a. Renal lithiasis.
 - b. Wegener granulomatosis.
 - c. FSGS.
 - d. Rapidly progressive glomerulonephritis.
 - e. Membranous glomerulonephritis.
 - f. Fanconi syndrome.
 - g. Acute renal failure.
 - h. Renal glucosuria, Fanconi syndrome.
 - i. Alport syndrome.

Chapter 9

- 1. a. Underdevelopment of the liver.
 - b. It will produce a transient green color.
 - c. Yes, with severe acquired liver disease.
 - d. Tyrosine crystals; leucine crystals, bilirubin crystals.
 - e. Protect the specimen from light.

270 Answers to Case Studies and Clinical Situations

- 2. a. DNPH test.
 - b. Isovaleric acidemia.
 - c. Maple syrup urine disease.
 - d. p-nitroaniline test. Methylmalonic acidemia is present.
 - e. Yes, this reaction is associated with maple syrup urine disease.
 - f. MS/MS.
- 3. a. Renal lithiasis.
 - b. Impaired renal tubular reabsorption of cystine.
 - c. Lysine, arginine, ornithine.
 - d. They are more soluble than cystine.
 - e. Cyanide-nitroprusside test.
 - f. Cystinuria.
- 4. a. Yes.
 - b. Yes, uric acid crystals accumulating on the surface of the diaper could have an orange color.
 - c. Lesch-Nyhan disease.
 - d. Yes, the disease is inherited as a sex-linked recessive.
 - e. Hypoxanthine guanine phosphoribosyltransferase.
- a. Yes, the urine may contain homogentisic acid or melanin.
 - b. A transient blue reaction with homogentisic acid or a gray-black reaction with melanin.
 - c. Transient blue.
 - d. Yes, melanin will react with sodium nitroprusside.
- 6. a. Yes, the purple color could indicate the presence of indican in the urine.
 - b. Ferric chloride with chloroform extraction and amino acid chromatography.
 - c. Hartnup disease.
 - d. Good with proper dietary supplements.
- 7. a. Porphyria.
 - b. No, the Watson-Schwartz test will only detect porphobilinogen, and the blockage in the heme pathway may be at another location, producing a product requiring fluorescent testing.
 - c. Yes, if the accumulated ALA is first converted to porphobilinogen using acetylacetone.
- a. Fructose.
 - b. Parenteral feeding.

Chapter 10

- 1. a. Cerebral hemorrhage because of the presence of erythrophagocytosis, even distribution of blood, and patient's history.
 - b. No, they would be consistent with peripheral blood entering the CSF.
 - c. No, they are consistent with the percentages seen in peripheral blood.
 - d. Hemosiderin granules and hematoidin crystals.
 - e. Accidental bone marrow puncture.
- 2. a. India ink preparation.
 - b. Cryptococcus meningitis.
 - c. Immunologic testing for Cryptococcus.
 - d. Rheumatoid factor.
 - e. Acid-fast staining and culture.

- f. Noticeable oligoclonal bands in both the CSF and
- 3. a. CSF/serum albumin index = 6.7
 - b. Yes
 - c. IgG index = 1.5
 - d. Immunoglobulin synthesis within the CNS.
 - e. Multiple sclerosis.
 - f. Oligoclonal banding only in the CSF.
 - g. Myelin basic protein.
- 4. a. Viral, fungal, tubercular meningitis.
 - b. No, this only applies to fungal meningitis.
 - c. Yes, lymphocytes are increased in viral meningitis.
 - d. Yes, a CSF lactate level of 25 mg/dL would aid in confirming viral meningitis.
- 5. a. Stain precipitate is being confused with gram-positive cocci.
 - b. Differentials are being reported from the counting chamber.
 - c. The albumin is contaminated.
 - d. The specimens are not being promptly delivered to the laboratory.

Chapter 11

- 1. a. Sperm concentration, motility, and morphology.
 - b. 21,000,000; no
 - c. 1,800,000; no
 - d. Yes. The normal sperm concentration is 20 to 60 million/mL. Spermatid counts over 1 million are considered abnormal. Both of these abnormal results and the abnormal motility are related to defects in sperm maturation.
- a. Male antisperm antibodies may form following vasovasectomy procedures.
 - b. The MAR test and the immunobead test.
 - c. The MAR test detects the presence of IgG male sperm antibodies. The Immunobead test delineates the areas of the sperm (head, tail, neck) that are affected by the antibodies.
 - d. Clumping, ovuum penetration, and motility.
- 3. The specimen contains urine, which is toxic to sperm, therefore decreasing viability.
- 4. The specimen was improperly collected, and the first part of the ejaculation was lost.
- 5. a. Yes, there is insufficient prostatic fluid present.
 - b. Zinc, citrate, and acid phosphatase.
 - c. Sperm motility is severely affected.
- 6. a. Acid phosphatase and seminal gycoprotein30 tests.
 - b. Microscopic examination for the presence of sperm.

Chapter 12

- 1. a. Sterile, heparinized tube, liquid EDTA tube, nonanticoagulated tube.
 - b. MSU crystals seen in gout.
 - c. Highly birefringent, needle-shaped crystals under polarized light that turn yellow when aligned with the slow vibration of compensated polarized light.

- d. Infection is frequently a complication of severe inflam-
- 2. a. WBC diluting fluid containing acetic acid was used.
 - b. Normal, hypotonic, or saponin-containing saline should be used.
 - c. Crystal-induced inflammatory and septic.
 - d. Gram stain and culture, crystal examination.
- 3. a. Noninflammatory.
 - b. Hydroxyapatite crystals.
 - c. The normal glucose result is consistent with noninflammatory arthritis.
- 4. a. Fibrinogen.
 - b. EDTA or heparinized tube.
 - c. No, the bacteria will be trapped in the clot.

Chapter 13

- 1. a. Pleural fluid.
 - b. Transudate, because all the test results are consistent with those of a transudate.
 - c. Pleural fluid:serum ratios of cholesterol and bilirubin.
- 2. a. Pneumonia.
 - b. Chest tube drainage.
- 3. a. 1.6
 - b. Transudate. The SAAG is above 1.1
 - c. Hepatic disorder.
- a. To differentiate between cirrhosis and peritonitis; cirrhosis.
 - b. Pancreatitis or gastrointestinal perforation; alkaline phosphatase.
 - c. Rupture or accidental puncture of the bladder.
 - d. To detect the presence of gastrointestinal (CEA) and ovarian (CA 125) cancers.
- The patient has been a victim of blunt trauma and the physician wants to determine if abdominal bleeding is occurring; abdominal bleeding.
- 6. Thyroid profile; CA 125.

Chapter 14

- 1. a. Yes.
 - b. FLM
 - c. The level of phosphatidylglycerol present in the fetal lungs.
 - d. Phosphatidylglycerol is essential for FLM, and levels do not always parallel lecithin levels in fetuses of diabetic mothers.
- 2. a. A neural tube disorder such as spina bifida or anencephaly.
 - b. An acetylcholinesterase level.
 - c. The amniotic fluid specimen contains blood.

- Increased, because lecithin bound to dye decreases polarization.
- 4. The results may be falsely decreased because some of the phospholipids may be sedimented.
- 5. a. False-positive result.
 - b. False-positive result.
 - c. No effect.
 - d. False-positive result.
- б. a. False-positive result.
 - b. False-positive result.
 - c. False-postive or test interference.
 - d. No effect.
- 7. The specimen was exposed to light, possible wrong specimen sent.

Chapter 15

- 1. a. Secretory diarrhea.
 - b. Stool culture.
 - c. **Probable**: Salmonella, Shigella, Campylobactor, Yersinia, E. coli; **Improbable**: Staphylococcus, Vibrio.
 - d. Osmotic diarrhea.
- 2. a. Microscopic examination for fecal fats.
 - b. Neutral fats stain directly and appear as large, orangered droplets; soaps and fatty acids appear as smaller orange-red droplets after pretreatment of the specimen with heat and acetic acid.
 - c. Quantitative fecal fat test.
 - d. Bulky and frothy.
 - e. Muscle fiber screening and the gelatin test for trypsin.
 - f. Muscle fiber: failure to include red meat in the diet; gelatin test: intestinal degradation of trypsin or the presence of trypsin inhibitors.
 - g. Chymotrypsin or elastase I.
- a. Patient Number One: gastric reflux medication containing bismuth may produce black stools; Patient Number Two: medications such as aspirin and other NSAIDs may cause gastric bleeding; Patient Number Three: red meat was not avoided for 3 days prior to sample collection.
 - b. Provide dietary and medication instructions to patients.
 - c. The Hemoccult ICT immunochemical test.
- 4. a. The APT test cannot be performed because the hemoglobin is already denatured.
 - b. The pH will be low because increased carbohydrates are available for bacterial metabolism.
 - c. The infant had ingested maternal blood.
 - d. Yes, adequate carbohydrates are not present, and fats are being metabolized for energy.



Answers to Study Questions

Chapter I

1.	C
2.	Α
3.	D

- 4. A
- 5. B
- 6. A
- 7. C
- 8. D
- 9. D
- 10. C
- 11. B 12. A
- 13. B
- 14. D
- 15. A
- 16. B
- 17. C
- 18. C
- 19. A 20. B
- 21. D
- 22. B
- 23. A
- 24. C
- 25. D

Chapter 2

- 1. B
- 2. D
- 3. C
- 4. D
- 5. A
- 6. B 7. C
- 8. D
- 9. B
- 10. A
- 11. C
- 12. D
- 13. A
- 14. B 15. B
- 16. D
- 17. B
- 18. A Inulin,
 - B Creatinine, B – Cystatin C,
 - $A {}^{125}$ I-iothalmate

- 19. B
- 20. 69 mL/min
- 21. A
- 22. C
- 23. B 24. D
- 25. A
- 26. C
- 27. B
- 28. C
- 29. +0.5
- 30. D
- 31. 600 mL/min
- 32. C
- 33. B

Chapter 3

- 1. C 2. B
- 3. A
- 4. B
- 5. True
- 6. C
- 7. C 8. C
- 9. D
- 10. C
- 11. C
- 12. A
- 13. A 14. False
- 15. B
- 16. B
- 17. C 18. C
- 19. A
- 20. B
- 21. D

Chapter 4

- 1. A 2. D
- 3. A
- 4. D
- 5. C 6. D
- 7. B
- 8. A 9. A

- 10. C
- 11. D
- 12. False 13. B
- 14. B
- 15. B
- 16. C 17. D
- 18. A
- 19. D
- 20. B 21. B
- 22. B
- 23. False 24. D
- 25. D

- 26. A

- Chapter 5
 - 1. C
 - 2. B 3. A
- 4. C
- 5. D 6. A
- 7. D
- 8. B 9. D
- 10. 2, 1, 2, 3, 1, 2, 3
- 11. B 12. A
- 13. B
- 14. D 15. C
- 16. C
- 17. A
- 18. C 19. B
- 20. B
- 21. C
- 22. A 23. B
- 24. C
- 25. B 26. A
- 27. D 28. A
- 29. C 30. 1, 2, 1, 2, 1, 2, 1, 1, 2

- 31. B
- 32. A
- 33. 2, 3, 4, 1
- 34. A
- 35. D 36. C
- 37. A
- 38. C
- 39. D
- 40. B 41. C
- 42. A 43. B
- 44. D
- 45. C 46. B
- 47. C 48. C
- 49. C 50. C

Chapter 6

- 1. C
- 2. D
- 3. D
- 4. C
- 5. A 6. B
- 7. C
- 8. D
- 9. C 10. D
- 11. D
- 12. A 13. C
- 14. B
- 15. C
- 16. A 17. D
- 18. D
- 19. B 20. C
- 21. A
- 22. B
- 23. C
- 24. D 25. D
- 26. B
- 273

10. D

11. C

9. C

10. C

274 Answers to Stud	ly Questions		
28. A	12. C	11. D	29. D
29. B	13. D	12. D	30. B
30. C	14. A	13. A	31. C
31. C	15. A	14. B	32. A
32. D	16. A	15. C	33. B
33. D	17. B	16. D	
34. B	18. D	17. D	
35. A	19. A	18. D	Chapter 12
36. C	20. D	19. B	1. B
37. C		20. D	2. B
38. D	Chapter 9	21. A	3. A
39. A	Chapter 7	22. D	4. B
40. C	1. A	23. D	5. B, C, B, A, D, B, D
41. D	2. C	24. B	6. A
42. A	3. B	25. C	7. C
43. A	4. D	26. A	8. B
44. C	5. A	27. C	о. в 9. D
45. D	6. D	28. A	
46. C	7. A	29. C	10. C 11. A
47. 4, 3, 5, 1	8. B	30. D	
48. 3, 5, 2, 6, 4	9. B	31. C	12. A
49. 4, 8, 7, 6, 1, 5, 3	10. D	32. B	13. C
50. 3, 5, 2, 1, 7, 4	11. D	33. A	14. A
30. 3, 3, 2, 1, 7, 1	12. D	34. C	15. B
Chantan 7	13. C	35. C	16. B
Chapter 7	14. A	36. B	17. A
1. D	15. B	30. B	18. D
2. D	16. C	Charter II	19. C
Z. D			
		Chapter II	20. C
3. D 4. B	17. A	1. C	21. D
3. D 4. B	17. A 18. D	-	21. D 22. A
3. D 4. B 5. 2, 1, 2, 3, 2, 1	17. A 18. D 19. B, A, B, B, A	1. C	21. D 22. A 23. D
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D	17. A 18. D 19. B, A, B, B, A 20. B	1. C 2. D	21. D 22. A 23. D 24. A
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B	17. A 18. D 19. B, A, B, B, A 20. B 21. D	1. C 2. D 3. B	21. D 22. A 23. D
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B	1. C 2. D 3. B 4. C	21. D 22. A 23. D 24. A
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A	1. C 2. D 3. B 4. C 5. D	21. D 22. A 23. D 24. A 25. B
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D 10. C	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A 24. B	1. C 2. D 3. B 4. C 5. D 6. A	21. D 22. A 23. D 24. A
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D 10. C 11. D	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A 24. B 25. A	1. C 2. D 3. B 4. C 5. D 6. A 7. B	21. D 22. A 23. D 24. A 25. B
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D 10. C 11. D 12. 1, 4, 3, 2, 1	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A 24. B 25. A 26. D	1. C 2. D 3. B 4. C 5. D 6. A 7. B 8. D	21. D 22. A 23. D 24. A 25. B
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D 10. C 11. D 12. 1, 4, 3, 2, 1 13. C	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A 24. B 25. A 26. D 27. D	1. C 2. D 3. B 4. C 5. D 6. A 7. B 8. D 9. C	21. D 22. A 23. D 24. A 25. B Chapter 13
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D 10. C 11. D 12. 1, 4, 3, 2, 1 13. C 14. D	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A 24. B 25. A 26. D 27. D 28. D	1. C 2. D 3. B 4. C 5. D 6. A 7. B 8. D 9. C 10. B	21. D 22. A 23. D 24. A 25. B Chapter 13 1. C 2. D
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D 10. C 11. D 12. 1, 4, 3, 2, 1 13. C 14. D 15. B	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A 24. B 25. A 26. D 27. D 28. D 29. C	1. C 2. D 3. B 4. C 5. D 6. A 7. B 8. D 9. C 10. B 11. B	21. D 22. A 23. D 24. A 25. B Chapter 13 1. C 2. D 3. A
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D 10. C 11. D 12. 1, 4, 3, 2, 1 13. C 14. D 15. B 16. 4, 1, 3, 2	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A 24. B 25. A 26. D 27. D 28. D 29. C 30. B	1. C 2. D 3. B 4. C 5. D 6. A 7. B 8. D 9. C 10. B 11. B 12. C	21. D 22. A 23. D 24. A 25. B Chapter 13 1. C 2. D 3. A 4. D
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D 10. C 11. D 12. 1, 4, 3, 2, 1 13. C 14. D 15. B	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A 24. B 25. A 26. D 27. D 28. D 29. C 30. B 31. D	1. C 2. D 3. B 4. C 5. D 6. A 7. B 8. D 9. C 10. B 11. B 12. C 13. A	21. D 22. A 23. D 24. A 25. B Chapter 13 1. C 2. D 3. A 4. D 5. C
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D 10. C 11. D 12. 1, 4, 3, 2, 1 13. C 14. D 15. B 16. 4, 1, 3, 2 17. False	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A 24. B 25. A 26. D 27. D 28. D 29. C 30. B	1. C 2. D 3. B 4. C 5. D 6. A 7. B 8. D 9. C 10. B 11. B 12. C 13. A 14. A	21. D 22. A 23. D 24. A 25. B Chapter 13 1. C 2. D 3. A 4. D 5. C 6. D
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D 10. C 11. D 12. 1, 4, 3, 2, 1 13. C 14. D 15. B 16. 4, 1, 3, 2	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A 24. B 25. A 26. D 27. D 28. D 29. C 30. B 31. D 32. D, F, A, E, C	1. C 2. D 3. B 4. C 5. D 6. A 7. B 8. D 9. C 10. B 11. B 12. C 13. A 14. A 15. B	21. D 22. A 23. D 24. A 25. B Chapter 13 1. C 2. D 3. A 4. D 5. C 6. D 7. B, A, A, A, B, B, A, B
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D 10. C 11. D 12. 1, 4, 3, 2, 1 13. C 14. D 15. B 16. 4, 1, 3, 2 17. False	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A 24. B 25. A 26. D 27. D 28. D 29. C 30. B 31. D	1. C 2. D 3. B 4. C 5. D 6. A 7. B 8. D 9. C 10. B 11. B 12. C 13. A 14. A 15. B 16. D	21. D 22. A 23. D 24. A 25. B Chapter 13 1. C 2. D 3. A 4. D 5. C 6. D 7. B, A, A, A, B, B, A, B 8. B
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D 10. C 11. D 12. 1, 4, 3, 2, 1 13. C 14. D 15. B 16. 4, 1, 3, 2 17. False Chapter 8	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A 24. B 25. A 26. D 27. D 28. D 29. C 30. B 31. D 32. D, F, A, E, C	1. C 2. D 3. B 4. C 5. D 6. A 7. B 8. D 9. C 10. B 11. B 12. C 13. A 14. A 15. B 16. D 17. B	21. D 22. A 23. D 24. A 25. B Chapter 13 1. C 2. D 3. A 4. D 5. C 6. D 7. B, A, A, A, B, B, A, B 8. B 9. B 10. C 11. D
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D 10. C 11. D 12. 1, 4, 3, 2, 1 13. C 14. D 15. B 16. 4, 1, 3, 2 17. False Chapter 8 1. B	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A 24. B 25. A 26. D 27. D 28. D 29. C 30. B 31. D 32. D, F, A, E, C	1. C 2. D 3. B 4. C 5. D 6. A 7. B 8. D 9. C 10. B 11. B 12. C 13. A 14. A 15. B 16. D 17. B 18. B	21. D 22. A 23. D 24. A 25. B Chapter 13 1. C 2. D 3. A 4. D 5. C 6. D 7. B, A, A, A, B, B, A, B 8. B 9. B 10. C
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D 10. C 11. D 12. 1, 4, 3, 2, 1 13. C 14. D 15. B 16. 4, 1, 3, 2 17. False Chapter 8 1. B 2. C	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A 24. B 25. A 26. D 27. D 28. D 29. C 30. B 31. D 32. D, F, A, E, C Chapter 10 1. B	1. C 2. D 3. B 4. C 5. D 6. A 7. B 8. D 9. C 10. B 11. B 12. C 13. A 14. A 15. B 16. D 17. B 18. B 19. A	21. D 22. A 23. D 24. A 25. B Chapter 13 1. C 2. D 3. A 4. D 5. C 6. D 7. B, A, A, A, B, B, A, B 8. B 9. B 10. C 11. D
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D 10. C 11. D 12. 1, 4, 3, 2, 1 13. C 14. D 15. B 16. 4, 1, 3, 2 17. False Chapter 8 1. B 2. C 3. B	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A 24. B 25. A 26. D 27. D 28. D 29. C 30. B 31. D 32. D, F, A, E, C Chapter 10 1. B 2. C 3. B 4. A	1. C 2. D 3. B 4. C 5. D 6. A 7. B 8. D 9. C 10. B 11. B 12. C 13. A 14. A 15. B 16. D 17. B 18. B 19. A 20. C	21. D 22. A 23. D 24. A 25. B Chapter 13 1. C 2. D 3. A 4. D 5. C 6. D 7. B, A, A, A, B, B, A, B 8. B 9. B 10. C 11. D 12. D
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D 10. C 11. D 12. 1, 4, 3, 2, 1 13. C 14. D 15. B 16. 4, 1, 3, 2 17. False Chapter 8 1. B 2. C 3. B 4. C	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A 24. B 25. A 26. D 27. D 28. D 29. C 30. B 31. D 32. D, F, A, E, C Chapter 10 1. B 2. C 3. B	1. C 2. D 3. B 4. C 5. D 6. A 7. B 8. D 9. C 10. B 11. B 12. C 13. A 14. A 15. B 16. D 17. B 18. B 19. A 20. C 21. A	21. D 22. A 23. D 24. A 25. B Chapter 13 1. C 2. D 3. A 4. D 5. C 6. D 7. B, A, A, A, B, B, A, B 8. B 9. B 10. C 11. D 12. D 13. D
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D 10. C 11. D 12. 1, 4, 3, 2, 1 13. C 14. D 15. B 16. 4, 1, 3, 2 17. False Chapter 8 1. B 2. C 3. B 4. C 5. B	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A 24. B 25. A 26. D 27. D 28. D 29. C 30. B 31. D 32. D, F, A, E, C Chapter 10 1. B 2. C 3. B 4. A	1. C 2. D 3. B 4. C 5. D 6. A 7. B 8. D 9. C 10. B 11. B 12. C 13. A 14. A 15. B 16. D 17. B 18. B 19. A 20. C 21. A 22. C	21. D 22. A 23. D 24. A 25. B Chapter 13 1. C 2. D 3. A 4. D 5. C 6. D 7. B, A, A, A, B, B, A, B 8. B 9. B 10. C 11. D 12. D 13. D 14. C
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D 10. C 11. D 12. 1, 4, 3, 2, 1 13. C 14. D 15. B 16. 4, 1, 3, 2 17. False Chapter 8 1. B 2. C 3. B 4. C 5. B 6. A	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A 24. B 25. A 26. D 27. D 28. D 29. C 30. B 31. D 32. D, F, A, E, C Chapter 10 1. B 2. C 3. B 4. A 5. C	1. C 2. D 3. B 4. C 5. D 6. A 7. B 8. D 9. C 10. B 11. B 12. C 13. A 14. A 15. B 16. D 17. B 18. B 19. A 20. C 21. A 22. C 23. A	21. D 22. A 23. D 24. A 25. B Chapter 13 1. C 2. D 3. A 4. D 5. C 6. D 7. B, A, A, A, B, B, A, B 8. B 9. B 10. C 11. D 12. D 13. D 14. C 15. B
3. D 4. B 5. 2, 1, 2, 3, 2, 1 6. D 7. B 8. C 9. D 10. C 11. D 12. 1, 4, 3, 2, 1 13. C 14. D 15. B 16. 4, 1, 3, 2 17. False Chapter 8 1. B 2. C 3. B 4. C 5. B 6. A 7. C	17. A 18. D 19. B, A, B, B, A 20. B 21. D 22. B 23. A 24. B 25. A 26. D 27. D 28. D 29. C 30. B 31. D 32. D, F, A, E, C Chapter 10 1. B 2. C 3. B 4. A 5. C 6. B, B, A, A	1. C 2. D 3. B 4. C 5. D 6. A 7. B 8. D 9. C 10. B 11. B 12. C 13. A 14. A 15. B 16. D 17. B 18. B 19. A 20. C 21. A 22. C 23. A 24. A	21. D 22. A 23. D 24. A 25. B Chapter 13 1. C 2. D 3. A 4. D 5. C 6. D 7. B, A, A, A, B, B, A, B 8. B 9. B 10. C 11. D 12. D 13. D 14. C 15. B 16. A

27. D 28. B 19. B

20. B

21. B	10. A	Chapter 15	16. False
22. C	11. C		17. C
23. B	12. B	1. C	18. C
24. A	13. True	2. A	19. C
25. D	14. A	3. C	20. D
	15. B	4. B	21. B
Chapter 14	16. True	5. D	22. A
Chapter 14	17. C	6. D	23. C
1. B	18. True	7. D	24. B
2. C	19. C	8. C	25. B
3. A	20. B	9. D	26. A
4. C	21. D	10. C	27. B
5. B	22. B	11. A	28. B
6. A	23. 1, 4, 2, 3	12. C	29. A
7. D	24. False	13. D	30. C
8. C	25. D	14. B	31. C
9. 2, 4, 1, 3	26. A	15. B	32. B



AAB	American Association of Bioanalysis	DBDH	Diisopropyl benzene dihydroperoxide
AABB	American Association of Blood Banks	DI	Diabetes insipidus
AAFP	American Academy of Family Physicians	DIDNTB	(3',3", diodo 4',4"-dihydroxy-5",5"-dinitro-
A:C	Albumin:creatinine		phenyl)-3,4,5,6-tetra-bromo-sulphonphtalein
ACE	Angiotensin converting enzyme	DNPH	2,4-dinitrophenylhydrazine
AChE	Acetylcholinesterase	EDS	Early dumping syndrome
ADA	Adenosine deaminase	EDTA	Ethylenediaminetetracetic acid
ADH	Antidiuretic hormone	ELISA	Enzyme-linked immunoabsorbent assay
AER	Albumin excretion rate	EPS	Expressed prostatic secretions
AFP	Alpha-fetoprotein	EU	Ehrlich unit
AGN	Acute glomerulonephritis	FAH	Fumarylacetoacetate hydrolase
AHG	Antihuman globulin	FDA	Food and Drug Administration
AIDS	Acquired immunodeficiency syndrome	FEP	Free erythrocyte protoporphyrin
AIN	Acute interstitial nephritis	FISH	Fluorescence in situ hybridization
AjBW	Adjusted body weight	FLM	Fetal lung maturity
ALA	α-Aminolevulinic acid	FOBT	Fecal occult blood testing
ANA	Antinuclear antibody	FSGS	Focal segmental glomerulosclerosis
ANCA	Antineutrophilic cytoplasmic antibody	FTA-ABS	Fluoresent treponemal antibody-absorption
AOA	American Osteopathic Association	GALT	Galactose-1-phosphate uridyl transferase
API	American Proficiency Institute	GFR	Glomerular filtration rate
APR	Auto particle recognition	GI	Gastrointestinal
ARF	Acute renal failure	GTT	Glucose tolerance test
ART	Assisted reproductive technology	H^+	Titratable acid/hydrogen ion
ASHI	American Society of Histocompatibility and	H_2PO_4	Hydrogen phosphate ion
	Immunogenetics	Hb	Hemoglobin
ASO	Antistreptolysin O	HBV	Hepatitis B virus
ATN	Acute tubular necrosis	hCG	Human chorionic gonadotropin
BAL	Bronchoalveolar lavage	HCO ₃ -	Bicarbonate ion
BAT	Bacterial antigen test	HDN	Hemolytic disease of the newborn
BSI	Body substance isolation	5-HIAA	5-Hydroxyindoleacetic acid
BUN	Blood urea nitrogen	HIV	Human immunodeficiency virus
CAP	College of American Pathologists	HLA-B12	Human leukocyte antigen-B12
CASA	Computer-assisted semen analysis	HOD	Harmonic oscillation densitometry
CDC	Centers for Disease Control and Prevention	hpf	High-power field
CEA	Carcinoembryonic antigen	IBS	Irritable bowel syndrome
CHP	Chemical hygiene plan	IBW	Ideal body weight
CLIA	Clinical Laboratory Improvement Amendments	IEF	Isoelectric focusing
CLSI	Clinical and Laboratory Standards Institute	IEM	Inborn error of metabolism
CMS	Centers for Medicare and Medicaid Services	IFE	Immunofixation electrophoresis
CNS	Central nervous system	iFOBT	Immunochemical fecal occult blood test
COC	Chain of custody	IgA	Immunoglobulin A
COLA	Commission on Laboratory Assessment	IgG	Immunoglobulin G
CPPD	Calcium pyrophosphate	IgM	Immunoglobulin M
CQI	Continuous quality improvement	IOM	Institute of Medicine
CSF	Cerebrospinal fluid	IOP	Improving organizational performance
CTAB	Cetyltrimethylammonium bromide	IVF	In vitro fertilization
Cu ₂ O	Cuprous oxide	JCAHO	Joint Commission on Accreditation of Health-
CUSO ₄	Copper sulfate		care Organizations
CV	Coefficient of variation	LBC	Lamellar body count
DCT	Distal convoluted tubule	LD	Lactate dehydrogenase

278 Abbreviations

LE	Leukocyte esterase	PPMT	Pre- and post-massage test
LED	Light-emitting diode	PSP	Phenolsulfonphthalein
LIS	Laboratory information system	PT	Proficiency testing
lpf	Low-power field	QA	Quality assurance
L/S	Lecithin-sphingomyelin ratio	QC	Quality control
MAR	Mixed agglutination reaction	RAAS	Renin-angiotensin-aldosterone system
MBP	Myelin basic protein	RBC	Red blood cell
MDRD	Modification of diet in renal disease	RCF	Relative centrifugal force
MESNA	Mercaptoethane sulfonate sodium	RDS	Respiratory distress syndrome
MoM	Multiples of the median	RF	Rheumatoid factor
mOsm	Milliosmole	RGE	Rapid gastric emptying
MPGN	Membranoproliferative glomerulonephritis	RPGN	Rapidly progressive (or crescentic) glomeru-
MS/MS	Tandem mass spectrophotometry		lonephritis
MSDS	Material Safety Data Sheet	RPM	Revolutions per minute
MSU	Monosodium urate (uric acid)	RPR	Rapid plasma reagin
MSUD	Maple syrup urine disease	RTE	Renal tubular epithelial (cells)
NaCl	Sodium chloride	SAAG	Serum-ascites albumin gradient
NCCLS	National Committee for Clinical Laboratory	SD	Standard deviation
	Standards	SKY	Fluorescent mapping spectral karyotyping
NFPA	National Fire Protection Association	SLE	Systemic lupus erythematosus
NH_4 +	Ammonium ion	SSA	Sulfosalicylic acid
NIRA	Near-infrared spectroscopy	TAT	Turnaround time
NRBC	Nucleated red blood cell	Tm	Maximal reabsorptive capacity/tubular reab-
OD	Optical density		sorptive maximum
OSHA	Occupational Safety and Health Administration	TmG	Maximal tubular reabsorption capacity for
PAH	<i>p</i> -aminohippuric acid		glucose
PB	Peripheral blood	TMB	3,3',5,5'-tetramethylbenzidine
PCT	Proximal convoluted tubule	TQM	Total quality management
PDCA	Plan-Do-Check-Act	UE ₃	Unconjugated estriol test
PDMAI	Plan-Design-Measure-Assess-Improve	UP	Universal Precautions
PDSA	Plan-Do-Study-Act	UTI	Urinary tract infection
PEP	Postexposure prophylaxis	VDRL	Veneral Disease Research Laboratory
PKU	Phenylketonuria	WBC	White blood cell
PM	Preventive maintenance	WHO	World Health Organization
PPE	Personal protective equipment	WSLH	Wisconsin State Laboratory of Hygiene
PPM	Provider-performed-microscopy		



accreditation The process by which a program or institution documents meeting established guidelines

accuracy Closeness of the measured result to the true value acrosomal cap Tip of a spermatozoa head, which contains enzymes for entry into an ovum

active transport Movement of a substance across cell membranes into the bloodstream by electrochemical energy

acute phase reactants Low-molecular-weight plasma proteins associated with infection and inflammation

adjusted body weight Actual body weight compared with ideal body weight to correct for weight attributed to fat and not muscle

aerosol Fine suspension of particles in air

afferent arteriole A small branch of the renal artery through which blood flows to the glomerulus of the kidney

albinism An inherited condition marked by decreased production of melanin

albuminuria Protein (albumin) in the urine

aldosterone A hormone that regulates reabsorption of sodium in the distal convoluted tubule

alimentary tract The digestive tract, including structures between the mouth and the anus

alkaptonuria Homogentisic acid in the urine caused by a failure to inherit the gene responsible for the production of homogentisic acid oxidase

aminoacidurias Disorders in which increased amino acids are present in the urine

amniocentesis Transabdominal puncture of the uterus and amnion to obtain amniotic fluid

amnion The membranous sac that contains the fetus and amniotic fluid

amyloid material A starchlike protein-carbohydrate complex that is deposited abnormally in tissue in some chronic disease states

andrology The study of diseases of the male reproductive organs

antiglomerular basement membrane antibody Autoantibody against alveolar and glomerular capillary basement membranes found in Goodpasture syndrome

anuria Complete stoppage of urine flow

arachnoid granulations Projections on the arachnoid membrane of the brain through which cerebrospinal fluid is reabsorbed

arthritis Inflammation of the synovial joints

arthrocentesis The puncture of a joint to obtain synovial fluid

ascites Abnormal accumulation of peritoneal fluid astrocytomas Tumors of the brain and spinal cord azotemia Increased nitrogenous waste products in the blood

bacterial endocarditis Inflammation of the endocardial membrane of the heart caused by bacterial infection

bacteriuria Bacteria in the urine

beta₂ microglobulin A subunit of the class I major compatibility antigens that enters the blood at a constant rate

bilirubin A bright yellow pigment produced in the degradation of heme

biohazardous Pertaining to a hazard caused by infectious organisms

birefringence The ability to refract light in two directions
 blood-brain barrier The barrier between the brain tissue and capillary blood that controls the passage of substances in the blood to the brain and cerebrospinal fluid

body substance isolation A guideline stating that all moist body substances are capable of transmitting disease

Bowman's capsule Part of the nephron that contains the glomerulus

bright-field microscopy A procedure by which magnified images appear dark against a bright background

bulbourethral glands Two small glands located on each side of the prostate gland

carcinogenic Capable of causing cancer

casts Elements excreted in the urine in the shape of renal

catheterized specimen A urine specimen collected by passing a sterile tube into the bladder

chain of custody Step-by-step documentation of the handling and testing of legal specimens

chain of infection A continuous link in the transmission of harmful microorganisms between a source and a susceptible host

chemical hygiene plan Protocol established for the identification, handling, storage, and disposal of all hazardous chemicals

chemical sieving Macroscopic screening of urine to determine the need for a microscopic examination

choroid plexuses A network of capillaries in the ventricle of the brain that produces cerebrospinal fluid

chylous material A milky lymphatic fluid that contains triglycerides and chylomicrons

cirrhosis Chronic liver disease that results in loss of liver cell function

clarity Transparency of urine, ranging from clear to turbid clue cell Squamous epithelial cell covered with the gramnegative bacteria, Gardnerella vaginalis

coefficient of variation Standard deviation expressed as a percentage of the mean

collecting duct Part of the nephron where the final concentration of urine takes place through the reabsorption of water

compensated polarized light Polarized light, using a compensator to separate light rays into slow- and fast-moving vibrations

constipation Infrequent production of feces that results in small, hard stools

continuous quality improvement An institutional program that focuses on customer satisfaction and expectations

control mean Average of all data points

control range Limit within which expected control values lie, usually plus or minus two standard deviations from the mean

countercurrent mechanism A selective urine concentration process in the ascending and descending loops of Henle

creatinine A substance formed by the breakdown of creatine during muscle metabolism

creatinine clearance A test used to measure the glomerular filtration rate

crenated Shrunken and irregularly shaped or notched cylindruria The presence of urinary casts

cystatin C Small protein produced at a constant rate by all nucleated cells

cystinosis An inherited recessive disorder that disrupts the metabolism of cystine

cystinuria Cystine in the urine that occurs as a result of a defect in the renal tubular reabsorption of amino acids

cystitis An inflammation of the bladder

cytogenetic analysis An analysis of cellular chromosomes dark-field microscopy Microscopic technique by which magnified images appear bright against a dark background

D-dimer A product of fibrinolysis

demyelination The destruction of the myelin sheath that protects a nerve

density Concentration of solutes present per volume of solution

diarrhea Watery stools

diarthroses Freely movable joints

disinfectant A substance that destroys microorganisms that is used on surfaces rather than the skin

distal convoluted tubule Part of the nephron between the ascending loop of Henle and the collecting duct where the final concentration of urinary filtrate begins

dysentery An inflammation of the intestines that is caused by microorganisms and results in diarrhea

dysmorphic Irregularly shaped

dyspnea Difficulty breathing

edema An accumulation of fluid in the tissues

efferent arteriole The small renal artery branch through which blood flows away from the glomerulus

effusion An accumulation of fluid between the serous membranes

endogenous procedure A test that uses a substance originating within the body

epididymis Small structure that forms the first part of the secretory duct of the testes

erythrophagocytosis Engulfment of red blood cells by macrophages *exogenous procedure* A test that requires a substance to be infused into the body

external quality control Commercial controls used to verify accuracy and reliability of patient test results

exudate Serous fluid effusion caused by conditions producing damage to the serous membranes

Fanconi syndrome A group of disorders marked by renal tubular dysfunction associated with some inherited and acquired conditions

fasting specimen The second voided urine specimen collected after fasting

ferritin A major storage form of iron found in the liver, spleen, and bone marrow

fetal lung maturity The presence of a sufficient amount of surfactant lipoproteins to maintain alveolar stability

first morning specimen The first voided urine specimen collected immediately upon arising; recommended screening specimen

flatus Gas expelled from the anus

fluorescent microscopy Microscopic technique by which naturally fluorescent substances or those that have been stained by a fluorescent dye produce an image when illuminated with a light of a specific wavelength

free water clearance A test to determine the ability of the kidney to respond to the state of body hydration

Froin syndrome Failure of spinal fluid in the spinal canal to mix with the cerebrospinal fluid in the ventricles, producing xanthochromic, rapidly clotting cerebrospinal fluid

fructosuria The presence of fructose in the urine galactosuria The presence of galactose in the urine gastrocolic fistula Abnormal passageway between the stomach and the colon

ghost cells Red blood cells that have lost their hemoglobin, leaving only the cell membrane; appear in hyposthenuric urine

glans The glandlike body at the tip of the penis

glomerular filtration rate The volume of plasma that is filtered by the glomerulus in a specified time

glomerulonephritis An inflammation of the glomerulus that results in impaired glomerular filtration

glomerulosclerosis The destruction of glomeruli by scarring and fibrin deposition

glomerulus Tuft of capillary blood vessels located in Bowman's capsule where filtration occurs

glucose tolerance specimens Fractional collection specimens; urine specimens are collected at the same time blood samples are drawn to compare the levels of glucose in blood and urine

glycogenesis The conversion of glucose to glycogen **glycogenolysis** The conversion of glycogen to glucose **glycosuria** Glucose in the urine (glucosuria)

granuloma Modular accumulation of inflammatory cells *Guillain-Barré syndrome* Autoimmune disorder that causes destruction of the myelin sheath that surrounds the peripheral nerves, resulting in loss of motor function

harmonic oscillation densitometry A method of measuring specific gravity by measuring the change in the frequency of a sound wave after it enters a solution

Hartnup disease A recessive inherited disorder marked by intestinal absorption abnormalities and renal aminoaciduria

hematiodin Yellow, crystalline substance that results from the distruction of red blood cells

hematuria Blood in the urine

hemoglobinuria Hemoglobin in the urine

hemolytic disease of the newborn Rh incompatibility between mother and fetus that can cause hemolysis of the fetal red blood cells

hemoptysis Blood in the sputum

hemosiderin An insoluble form of storage iron; a product of red blood cell hemolysis

hemothorax The accumulation of blood in the pleural cavity homocystinuria The presence of homocystine in the urine caused by an inherited autosomal recessive disorder

2-hour postprandial specimen Fractional collection specimen; urine specimen collected 2 hours after eating

hyaluronic acid Glycosaminoglycan found in synovial fluid that provides lubrication to the joints

hydramnios Excess amniotic fluid

hydrostatic pressure Pressure exerted by a liquid

hyperglycemia Elevated glucose levels in the blood

hypernatremia Elevated blood sodium levels

hypersthenuric Pertaining to urine specific gravity greater than the 1.010 of the glomerular filtrate

hyponatremia Decreased blood sodium levels

hyposthenuric Pertaining to urine specific gravity lower than the 1.010 of the glomerular filtrate

hypoxia Lack of oxygen

iatrogenic Pertaining to a condition caused by treatment, medications, or diagnostic procedures

ideal body weight Statistical calculation of body weight related to height

immune complexes Antigen-antibody combinationsinborn error of metabolism Failure to inherit the gene to produce a particular enzyme

indicanuria The presence of indican in the urine
infertility The inability to conceive

interference-contrast microscopy A procedure by which three-dimensional images of a specimen are obtained

internal quality control Electronic, internal, and procedural controls contained within the test system that ensures the reliability of the test system

interstitial Pertaining to spaces between tissue cellsinulin A fructose-derived substance that is filtered by the kidney and not reabsorbed or secreted and that can be used to measure the glomerular filtration rate

in vitro fertilization Fertilization between an ovum and a sperm performed in the laboratory

ischemia Deficiency of blood to a body area

isosthenuric Pertaining to urine specific gravity the same as the 1.010 of the glomerular filtrate

jaundice Yellow appearance of skin, mucous membranes, and eye sclera due to increased amounts of bilirubin in the blood

juxtaglomerular apparatus Specialized cells located on the afferent arteriole that regulate secretion of renin

ketonuria Ketones in the urine

labia The outer folds of the vagina

lactosuria The presence of lactose in the urine

lamellar body Organelle produced by type II pneumonocytes in the fetal lung that contain lung surfactants

Langerhans cells Pancreatic cells

lecithin Phospholipid that forms part of the cell wall used to determine fetal lung maturity

lecithin-sphingomyelin ratio A comparison of lung surfactants that is performed to determine fetal lung maturity

Lesch-Nyhan disease An inherited sex-linked recessive purine metabolism disorder marked by excess uric acid crystals in the urine

leukocyturia Leukocytes (white blood cells) in the urine liquefaction The conversion of solid or coagulated material to a liquid form

lithiasis The formation of renal calculi (kidney stones)
lithotripsy A procedure that uses ultrasonic waves to crush renal calculi

loop of Henle The U-shaped part of the renal tubule that consists of a thin descending limb and a thick ascending limb

lysosome Cellular organelle that contains digestive enzymes

macula densa Specialized cells located on the distal convoluted tubule that interact with the juxtaglomerular cells

malabsorption Impaired absorption of nutrients by the intestine

maldigestion Impaired digestion of foodstuffs

maple syrup urine disease An autosomal recessive trait that causes increased levels of the branched-chain amino acids, leucine, isoleucine, valine, and their ketone acids in the urine

Material Safety Data Sheet A document provided by the vendor or manufacturer of a chemical substance that describes the chemical's characteristics

maximal reabsorptive capacity The maximum reabsorption ability for a solute by renal tubules

meconium The dark-green mucus-containing stool formed by a fetus

medullary interstitium Spaces between the cells in the medulla of the kidney that contain highly concentrated fluid

medulloblastomas Malignant tumor of the fourth ventricle and cerebellum

melanoma A tumor of the melanogen-producing cells, which is frequently malignant

melanuria Increased melanin in the urine

melituria Increased urinary sugar

meninges Protective membranes around the brain and spinal cord

meningitis Inflammation of the meninges, frequently caused by microbial infection

mesothelial cells Cells that line the serous membranes *metabolic acidosis* A decrease in the blood pH caused by a metabolic increase in acidic elements

microalbuminuria Low levels of urine protein that are not detected by routine reagent strips

midstream clean-catch specimen Specimen collected in a sterile container after cleansing the glans penis or urinary meatus; the first portion of urine is voided into the toilet, the midportion is collected, and the remaining portion is voided into the toilet

mucin Glycoprotein found in mucus and in the skin, connective tissues, tendons, and cartilage

mucopolysaccharides Glycosaminoglycans that consist of a protein core with polysaccharide branches

mucopolysaccharidoses A group of genetic disorders marked by excess mucopolysaccharides in blood and urine

multiple myeloma Malignant disorder that results in infiltration of the bone marrow by plasma cells

myoglobin Iron-containing protein found in muscle tissue *myoglobinuria* Myoglobin in the urine

necrosis Death of cells

nephron A functional unit of the kidney that forms urine *nephropathy* Disease of the kidneys

nephrotic syndrome A renal disorder marked by massive proteinuria, lipiduria, and edema caused by disruption of the glomerular membrane

nocturia Excessive urination during the night

occult blood Blood that is not visible to the naked eye

Occupational Safety and Health Administration The government agency created to protect employees from potential health hazards in the workplace through the development and monitoring of regulations

oligoclonal bands Electrophoretic bands migrating in the gamma region that are present in cerebrospinal fluid and serum

oligohydramnios Decreased amniotic fluid oliguria A marked decrease in urine flow

oncotic pressure The osmotic pressure of a substance in solution caused by the presence of colloids

organic acidemias The accumulation of organic acids in the blood, mainly isovalaric, propionic, and methylmalonic acids

orthostatic proteinuria Increased protein in urine only when an individual is in an upright position

osmolar clearance The amount of plasma filtered each minute to produce a urine with the same osmolarity as plasma

osmolarity The osmotic pressure of a solution expressed in milliosmols per kilogram; it is affected only by the number of particles present

osmotic diarrhea An increased retention of water and solutes in the large intestine associated with malabsorption and maldigestion

osmotic gradient The difference in the concentration of substances on either side of a membrane

outcomes Results of the process to improve customer satisfaction

pancreatic insufficiency The decreased ability of the pancreas to secrete digestive enzymes

paracentesis Surgical puncture into the abdominal cavity to obtain peritoneal fluid

parietal membrane Serous membrane that lines the walls of the pleural, pericardial, and peritoneal cavities

passive transport Movement of molecules across a membrane by diffusion because of a physical gradient

pentosuria The presence of pentose sugars in the urine pericardiocentesis Surgical puncture into the pericardial cavity to obtain pericardial fluid

pericarditis An inflammation of the membranes enclosing the heart

peritoneal lavage Introduction and subsequent removal of fluid into the peritoneal cavity to detect the presence of abnormal substances

peritonitis An inflammation of the membranes that line the peritoneal cavity

peritubular capillaries The capillaries that surround the renal tubules

personal protective equipment Items used to protect the body from infectious agents

phase-contrast microscopy Procedure in which magnified images show varied intensities of light and dark and are surrounded by halos

phenylketonuria The presence of abnormal phenylalanine metabolites in the urine

phosphatidyl glycerol Phospholipid found in amniotic fluid that is used to confirm fetal lung maturity

pigmented villonodular synovitis Proliferation of synovial cells forming brown nodules, resulting in inflammation, pain, and hemorrhagic effusions

pleocytosis Increased numbers of normal cells in the cerebrospinal fluid

podocytes Epithelial cells of the inner lining of Bowman's capsule that contain footlike processes

polarizing microscopy A procedure in which magnified birefringent images appear bright or colored against a black background

polydipsia Excessive thirst

polyhydramnios Excessive amniotic fluid

polyuria Marked increase in urine flow

porphobilinogen Immediate precursor of the porphyrins involved in the synthesis of heme

porphyrias Disorders of porphyrin metabolism that are inherited or acquired

porphyrins Intermediate compounds in the synthesis of heme

porphyrinuria The presence of porphyrins in the urine postexposure prophylaxis Preventative treatment provided following exposure to a potentially harmful agent

postrenal proteinuria Increased protein in the urine caused by infections/inflammation that add protein to the urine after its formation

precision Reproducibility of a test result

- prerenal proteinuria Increased protein in the urine caused by factors affecting the plasma before it reaches the kidney
- *preventive maintenance* Checks on instruments and equipment on a regular schedule
- process System of what is done to the patient
- proficiency testing Performance of tests on specimens provided by an external monitoring agency
- prostate Muscular gland surrounding the male urethra protein error of indicators Indicators change color in the presence of protein at a constant pH
- proteinuria Protein in the urine (albuminuria)
- *proximal convoluted tubule* The nearest tubule to the glomerulus where reabsorption of essential substances begins
- pseudochylous material Milky effusion that does not contain chylomicrons
- purpura Small capillary hemorrhages
- pyelonephritis Infection of the renal tubules
- pyknotic Referring to a dense, round nucleus
- pyuria The presence of white blood cells (pus) in the urine quality assessment Methods used to guarantee quality patient care
- *quality control* Methods used to monitor the accuracy of procedures
- *quality system* The overall laboratory policies, procedures, processes, and resources to achieve quality test results
- *radioisotope* A substance that emits radiant energy
- ragocytes Neutrophils that contain ingested clumps of IgG random specimen Urine collected at any time without prior patient preparation
- **refractometry** Measurement of the light-bending capability of solutions
- **Reiter cells** Vacuolated macrophages containing ingested neutrophils associated with nonspecific arthritic inflammation
- *reliability* The ability to maintain both precision and accuracy
- *renal plasma flow* The volume of plasma passing through the kidneys per minute
- *renal proteinuria* Protein in the urine caused by impaired renal function
- **renal threshold** Plasma concentration of a substance at which active transport stops and increased amounts are excreted in the urine
- *renal tubular acidosis* The inability to produce an acidic urine in the presence of metabolic acidosis
- *renin* Proteolytic enzyme produced by the kidney that reacts with angiotensinogen to produce angiotensin to increase blood pressure
- **renin-angiotensin-aldosterone system** Regulates flow of blood to and within the kidneys by responding to changes in blood pressure and plasma sodium content
- **resolution** The ability to separate fine structures for visualization of detail
- *retinoblastomas* Malignant glioma of the retina seen in young children

- **Reye syndrome** Acute encephalopathy and liver infiltration seen in children following viral infections
- rhabdomyolysis Muscle destruction
- rheumatoid factor Immunoglobulin associated with rhematoid arthritis
- sarcoidosis Multisystem disease caused by infiltration of the organs by T lymphocytes and phagocytes that form granulomas in the tissues
- **secretory diarrhea** The increased secretion of water and electrolytes into the large intestine caused by bacterial enterotoxins
- semen Fluid-containing spermatozoa
- seminal vessels Two sac-like structures close to the prostate that produce the majority of the seminal fluid
- seminiferous tubules Tubules that produce or conduct semen
- **sentinel event** Unexpected death or permanent loss of function not caused by a patient's underlying condition
- **serous fluid** Fluid formed as a plasma ultrafiltrate that provides lubrication between the parietal and visceral serous membranes
- shift Abrupt change in the mean of a series of results
- *Sjögren syndrome* An autoimmune disorder associated with a defect in glandular production of moisture
- *specific gravity* The density of a solution compared with that of a similar volume of distilled water, influenced by both the number and size of the particles present
- spermatids Immature spermatozoa
- spermatozoa Sperm cells
- **sphingomyelin** Phospholipid found in amniotic fluid used to determine fetal lung maturity
- **standard deviation** Measurement statistic that indicates the average distance each data point is from the mean
- **Standard Precautions** Guideline describing personnel protective practices
- steatorrhea Excess fat in the feces
- stercobilinogen Substance derived from urobilinogen that is found in the feces and is oxidized to form urobilin forming the brown color of feces
- stool Fecal material discharged from the large intestine
- **subarachnoid space** The area between the arachnoid and pia mater membranes
- suprapubic aspiration The technique used to obtain sterile urine specimens for bacterial culture or cytologic examination, in which a sterile needle is introduced through the abdomen into the bladder
- **surfactants** Phospholipids secreted by type II pneumocytes to maintain alveolar integrity
- **syncytia** A group of cells with continuous adjoining cell
- synovial fluid Plasma ultrafiltrate that contains hyaluronic acid and provides lubrication of the joints
- **synoviocytes** Cells in the synovial membrane that secrete hyaluronic acid
- systemic lupus erythematosus Autoimmune disorder that affects the connective tissue and results in damage to organs, particularly the kidney and joints

- *Tamm-Horsfall protein* Mucoprotein found in the matrix of renal tubular casts
- thoracentesis Surgical puncture into the thoracic cavity to collect pleural fluid
- three-glass collection Urine specimen collected in three separate sterile containers; used to determine prostatic infectionthrombosis Formation of a blood clot
- *timed specimen* Urine specimen collected over an interval of time for a quantitative analysis of a urine chemical, usually a 24-hour collection
- *titratable acidity* Hydrogen ions in the urine that can be quantitated by titration with a base to a pH of 7.4
- **total quality management** Institutional policy to provide customer satisfaction
- *transudate* Serous effusion produced as a result of disruption of fluid production and regulation between the serous membranes
- *traumatic tap* Surgical puncture contaminated with capillary blood
- *trend* Gradual change in one direction of the mean of a control substance
- *tubular reabsorption* Substances moved from the tubular filtrate into the blood by active or passive transport
- *tubular secretion* The passage of substances from the blood in the peritubular capillaries to the tubular filtrate
- *tubulointerstitial disease* Renal disease that affects both the renal tubules and renal interstitium
- *turnaround time* Time from ordering a test through analysis in the laboratory to the charting of the report

- tyrosyluria The presence of tyrosine in the urine
- *Universal Precautions* Guideline stating that all patients are capable of transmitting blood-borne disease
- urinary meatus The external urinary opening
- *urinometry* An imprecise method for measuring urine specific gravity using a weighted float
- *urobilin* The oxidized form of urobilinogen that provides the brown color to feces
- *urobilinogen* A compound formed in the intestines by the bacterial reduction of bilirubin
- **urochrome** Yellow pigment produced by endogenous metabolism that imparts the yellow color to urine
- *uroerythrin* Pink pigment in urine derived from melanin metabolism that attaches to urates in the sediment
- vasa recta A network of capillaries that surrounds the loop of Henle
- vasectomy Surgical removal of all or part of the vas deferens for the purpose of male sterilization
- *vasopressin* Antidiuretic hormone that regulates reabsorption of water by the collecting ducts
- vasovasostomy Repair of a severed vas deferens to restore fertility
- visceral membrane The serous membrane covering the organs contained within a cavity
- viscosity The amount of resistance to flow in a liquid
- visicoureteral reflux Urine in the bladder passing back into the uterers
- *xanthochromia* Yellowish discoloration of the cerebrospinal fluid



Ammonium biurate crystals, 116f

Amniocentesis, 237

Index

Page numbers followed by f indicate figures; page numbers followed by t indicate tables.

A	Amnion, 235, 236f	Basement membrane, 14f
Abbott TDx analyzer, 241	Amniostat-FLM, 240	Bayer Multistix Pro 11 reagent strips, 61
Abnormal urine crystals, 116-121	Amniotic acetylcholinesterase (AChE), 239	Bence Jones protein, 57–58
ampicillin crystals, 119	Amniotic cavity, 236f	Benedict solution, 63
characteristics of, 117t	Amniotic fluid, 235–242	Berger disease. See Immunoglobulin A
cholesterol crystals, 116	chemical composition, 236	nephropathy
cystine crystals, 116	collection, 237	Beta-hydroxybutyric acid, production of from
liver disorders and, 118	color and appearance, 238	acetoacetic acid, 65f
radiographic dye crystals, 116-118	differentiating from maternal urine, 237	Bicarbonate, reabsorption of filtered, 18f
sulfonamide crystals, 118-119	function, 235	Bilirubin, 43, 54, 230
ACE. See Angiotensin converting enzyme	physiology of, 235-237	clinical significance of in urine, 68-69
Acetest procedure, 65	specimen collection, 237	conjugated, 68, 70, 248
Acetest tablets, 65	specimen handling and processing, 237-238	production of, 68
Acetone, production from acetoacetic acid, 65f	volume, 236	reaction interference in urine, 69
AChE. See Amniotic acetylcholinesterase	Amniotic sac, 236f	reagent strip reaction to in urine, 68
Acid-albumin test, 170	Amorphous phosphates, 115f	reagent strip summary, 69
Acid steatocrit procedure, 252	Amorphous urates, 114f	in urine, 68–69
Acidosis, 227	Ampicillin crystals, 119f	Bilirubin crystals, 119f
Active transport, 15	Amyloid material, 58	Bilirubinuria, 68
Acute interstitial nephritis, 151	Andrology, 199	Biliverdin, 69
Acute phase reactants, 57	Angiotensin converting enzyme (ACE), 14	Biohazardous material, 2
Acute poststreptococcal glomerulonephritis	Angiotensin II, 14	Biological hazards, 2–5
(AGN), 144	Antibodies, antisperm, 206-207	Biological waste, disposal of, 5, 8
Acute pyelonephritis, 150	Antidiuretic hormone (ADH), 17, 23, 31, 149	Birefringence, in polarized microscopy, 90
Acute renal failure (ARF), 152	Antiglomerular basement membrane antibody,	Birefringent, 216
Acute tubular necrosis (ATN), 149, 152	144	calcium pyrophosphate (CPPD) crystals, 217
Addis count, 82	Antihuman albumin antibody, 61	monosodium urate (MSU) crystals, 217f
Addis, Thomas, 30, 82	Antihuman globulin (AHG), 206	Blood
ADH. See Antidiuretic hormone	Antineutrophilic cytoplasmic antibody (ANCA),	clinical significance of in urine, 65-67
Adjusted body weight (AjBW), 21	145	reaction interference in urine, 67
ADVIA120 Hematology System, 181, 260t, 263	Antinuclear antibody (ANA), 228	reagent strip reaction to in urine, 67
ADVIA2120 Hematology System, 260t, 263	Antistreptolysin O (ASO), 144	reagent strip summary, 67
ADVIA Urinalysis Workcell System, 260t, 263	Anuria, 31	Blood-brain barrier, 178
Afferent arteriole, 13–14, 13f, 14f, 15f	Anus, 200f	Blood pH, 18
AHG. See Antihuman globulin	APT test	Blood urea nitrogen (BUN), 144
AIDS, 184, 229	fetal hemoglobin, for, 252-253	Body substance isolation (BSI), 3
Albinism, 163	procedure for, 253	Body surface area, nomogram for determining,
Albumin, 57	Arachnoid villus/granulation, 178f	21f
Albumin: Creatinine ratio, 61	ARF. See Acute renal failure	Bone marrow contamination, 185f
Albumin excretion (AER), 58	Arthritis, 211	Borrelia burgdorferi, 218
Albuminuria, 30	Arthrocentesis, 212	Bowman's capsule, 13-14, 13f, 17f
Aldosterone, 14, 17	Articular capsule, 212f	Bowman's space, 14f, 144
Alimentary tract, 246	Articular cartilage, 212f	Branched-chain amino acid disorders, 164-165
Alkaptonuria, 44, 164	Articulating bone, 212f	maple syrup urine disease, 164-165
Alpha-fetoprotein (AFP), 239	Assisted reproductive technology (ART), 199, 207	organic acidemias, 165
Alport syndrome, 147	ATN. See Acute tubular necrosis	Bright-field microscopy, 83, 87, 89
American Association of Bioanalysts, 208	AUTION MAX AX-4280, 262	Bright, Richard, 30
Amino acid disorders, 161–167	Automated microscopy, 260t, 262	Broad casts, 109-110
branched-chain, 164-165	Automated urinalysis systems, 260t, 263	Bronchoalveolar lavage (BAL), 265-266, 265f,
phenylalanine-tyrosine disorders, 161–164	Auto Particle Recognition (APR) software, 262	266f
alkaptonuria, 164	Azotemia, 152	Bryant, Thomas, 30
melanuria, 163		Bulbourethral gland, 199, 200f
phenylketonuria, 161–163	В	Burkitt lymphoma, 189f
tyrosyluria, 163	Bacillus subtilis, 162	
Aminoacidurias, 161	Bacteria, 100, 100f	C
Ammonium, 32t	Bacterial antigen test (BAT), 193	Calcium, 32t

Bacterial endocarditis, 229

Bacteriuria, 72

Calcium carbonate crystals, 116f

Calcium oxalate crystals, 115f

Calcium pyrophosphate (CPPD), 214–216	clot formation, 180	Constipation, 246
crystals, 217f	methodology, 181	Continuous quality improvement (CQI), 136-137
weakly birefringent, 216f	xanthochromatic supernatant, 180	Control mean, 132
Wright stained crystals, 216f	white blood cell count, 181	Control ranges, 132
Candida albicans, 100	Cerebrospinal protein, 189-191	Copper reduction test, 63-64
Carbohydrate disorders, 170	Cerebrum, 178f	Cortex, 13f
Casts	Cetyltrimethylammonium bromide (CTAB) test,	Cortical nephrons, 12
broad, 109-110	169	Countercurrent mechanisms, 17
composition and formation, 102-103	Chain of custody (COC), 37	CPPD. See Calcium pyrophosphate
fatty, 107, 107f, 108f	Chain of infection, 2, 2f	Creatinine, 20f, 32t
fiber resembling a, 120f	Chemical handling, 6	Creatinine clearance, 19, 21
granular, 108-109, 108f, 109f, 110f	Chemical hazards, 6–7	Critical results reporting, 135f
granular dirty brown, 106f	chemical hygiene plan, 6	Cryptococcus neoformans, 193, 265, 266
mixed cellular, 107-108	chemical labeling, 6-7	gram stain of, 194f
urine sediment, in, 102-110	handling of chemicals, 6	India ink preparation of, 193f
waxy, 109, 109f, 110f	Material Safety Data Sheets (MSDS), 7	Crystals, calcium oxalate, urine in, 113, 114f
Cell count, in cerebrospinal fluid, 180–182	spills, 6	CSF. See Cerebrospinal fluid
Cells, malignant, of hematological origin, 188	Chemical hygiene plan (CHP), 6	CTAB. See Cetyltrimethylammonium bromide test
Cells, nonpathologically significant, in	Chemical labeling, 6–7	CV. See Coefficient of variation
cerebrospinal fluid, 184	Chemical safety aids, 7f	Cyanide-nitroprusside test, 166
Centers for Disease Control and Prevention	Chemical sieving, 82. See also Urine, macroscopic	Cylindruria, 103
(CDC), 3-4, 136, 167, 194	screening of	Cystatin C, 21
Centers for Medicare and Medicaid Services	Chemical spills, 6	Cystine crystals, 116, 117f
(CMS), 136	Chemstrip, 54, 56, 67, 70	Cystine disorders, 166–167
Central nervous system (CNS), 178, 184	Chemstrip 101, 260t, 261	cystinosis, 166–167
Centrifugation, 83	Chemstrip Criterion II, 260t, 261	cystinuria, 166
Cerebellum, 178f	Chemstrip Super Automated Urine Analyzer,	homocystinuria, 167
Cerebrospinal fluid (CSF), 178–194	260t, 261–262	Cystitis, 149
abnormal protein values in, 190t	Chemstrip Urine Analyzer, 260t, 261	Cytocentrifuge recovery chart, 183t
appearance, 179	Cholesterol crystals, 116, 118f	Cytogenetic analysis, 235
appearance, clinical significance of, 180t	Chorion, 236f	Cytospin 3 cytocentrifuge, 182f
artificially induced plasma proteins in, 190	Choroid plexus, 178, 178f	c) ***
analyzers, 260t, 263	Choroidal cells, 187f	D
assay, 263	CHP. See Chemical hygiene plan	D-dimer test, 180
cell count, 180–182	Chronic glomerulonephritis, 145	Dark field microscopy, 91, 91f
cerebrospinal protein, 189–191	Chronic pyelonephritis, 150–151	Dekkers, Frederik, 30
electrophoresis, 191	Chylous material, 224, 224t	Deming, Edward, 137
fractions, 190–191	Ciliated columnar bronchial epithelial cells, 265,	Demyelination, 191
methodology, 190	266f	Diabetes, 246
chemistry tests on, 189–192	Clinical and Laboratory Standards Institute	differentiation between insipidus and mellitus,
collection and handling, 178–179	(CLSI), 30, 46, 82, 84, 128	33f
constituents, 183–189	Clinical Laboratory Improvement Amendments	gestational, 62
eosinophils, 184	'88 (CLIA), 135–136, 207, 259	insipidus, 31
lymphocytes, 184	Clinical laboratory safety, 1–8	mellitus, 31, 61
macrophages, 184	Clinitest procedure, 64	type 2, 62
malignant cells, 188–189	Clinitest reaction, 131, 170	Diabetic nephropathy, 147
monocytes, 184	Clinitest tablet, 63	Diarrhea, 246–248
neutrophils, 184	Clinitek 50, 259, 260f, 260t	altered motility, 247
nonpathologically significant cells, 184	Clinitek 200/200+, 259, 260t	common fecal tests for, 247t
contamination, corrections for, 182	Clinitek 500, 259, 260t, 261f	osmotic, 247
cytocentrifugation, 182–183	Clinitek Atlas, 259–260, 260t, 261f	secretory, 247
differential cell counts in specimen, 182–189	Clinitek Microalbumin reagent strips, 61, 259	Diarthroses, 211
elevated protein values, significance of, 190	Clinitek Status, 259, 260t	Diazo reaction, 68
flow of, 178f	Clue cell, 97	Disinfectant, 4
formation and physiology of, 178	CMS. See Centers for Medicare and Medicaid	Dissociation constant (pK), 75
glucose in, 191–192	Services	Distal convoluted tubule, 13, 13f
microbiology tests on, 192–193	CNS. See Central nervous system	Distal tubule, 15f
predominant cells seen in, 183t	COC. See Chain of custody	Documentation procedure review, 128f
quality control in cell counts, 182	Cockcroft, D. W., 21	DPNH test. See 2,4–Dinitrophenylhydrazine test
serologic testing on, 194–195	Coefficient of variation (CV), 132	Dura mater, 178, 178f
serum protein correlations and, 190t	Coffin lid crystals, 115f	Dysentery, 248
simulated procedure, 194	College of American Pathologists, 208	Dyspnea, 145
specimen collection tubes, 179f	Colligative property, 23	Dyspica, 113
teaching analysis of, 195	Colon carcinoma cells, 231f	E
total cell count, 181	Combur TestUX urine test strips, 261	Early dumping syndrome (EDS), 247
traumatic collection (tap), 179–180	Concentration factor, 83	Edema, 144
blood distribution in, 179–180	Concentration tests, 22	EDTA. See Ethylenediaminetetraacetic acid
capillary and tissue fragments, 186f	Conjugated bilirubin, 68, 70, 248	Edwin Smith Surgical Papyrus, 29
capitally and tissue flagilietits, 1001	Conjugated Dimubin, OO, 10, 270	Lawin Jilili Juigicai i apyrus, 29

287

Efferent arteriole, 13–14, 13f, 14f, 15f	neutral fat stain procedure, 250	Glomerular filtration, 13–14, 14f
Effusions, 213, 222	physiology, 245–246	calculated estimate, 20–22
pathological causes of, 222t	qualitative facts, 249–250	Glomerular filtration rate (GFR), 13, 19, 20,
Ehrlich reaction, 167	specimen collection, 248	21–22
Ehrlich-reactive compounds, 70	split fat stain procedure, 250	Glomerular filtration tests, 18-22
Ehrlich reagent, 70, 163	Ferric chloride tube test, 162	clinical significance of, 20
Ehrlich test, 167	Ferritin, 66	creatinine clearance, 19
Ehrlich tube test, 70–71	Fetal distress	inulin clearance, 19
Ehrlich units (EU), 70	neural tube defects, 239	procedure, 19-20
Ehrlich's aldehyde reaction, 70	tests for, 238-239	Glomerular pressure, 14
Ejaculatory duct, 200f	Fetal Lung Maturity (FLM), 237-242	Glomerular proteinuria, 58
Electrical hazards, 7–8	tests for, 239–242	Glomerulonephritis, 105-106, 144-145
Electrophoresis, 191	amniostat-FLM, 240	acute poststreptococcal, 144
ELISA. See Latex agglutination and	foam stability, 240–241	chronic, 145
enzyme–linked immunosorbent assay	lamellar bodies and optical density, 241-242	Goodpasture syndrome, 144–145
Endogenous procedure, 19	lecithin-sphingomyelin (LS) ratio, 240	membranoproliferative, 145
Endothelium, 14f	microviscosity:fluorescence polarization	membranous, 145
Enterobius vermicularis, 100	assay, 241	rapidly progressive (crescentic), 144
Environmental control, 3	Fetal Lung Maturity II Calibrators, 241	Glomerulus, 13, 13f, 15f, 22
Eosinophils, 95, 95f, 184, 186f, 265	Fetal Lung Maturity II (FLMII) assay, 241	Gloves, 3
Ependymal cells, 188f	Fetal maturity, tests for, 236t, 239-242	Glucose
Epididymis, 199, 200f	Fetal well-being, tests for, 236t	cerebrospinal fluid, in, 191–192
Epinephrine, 62	Fetus, 236f	clinical significance of in urine, 62
Epithelial cells, 97f	Fiber, 120f, 121f	copper reduction test for in urine, 63–64
casts, 106–107	Fibrous capsule, 212f	reaction interference in urine, 63
summary of, 101	Fire extinguishers, 8t	reagent strip reaction to in urine, 62–63
transitional, 98f	Fire hazards, 8	reagent strip summary, 63
in urine, 95–100	Fires, 8t	in urine, 61–64
Erythrocytes, 265	Fishberg concentration test, 22	Glucose oxidase
Escherichia coli, 67	5-Hydroxyindoleacetic acid (HIAA), 166	Clinitest reaction summary, and, 64
Ethylenediaminetetraacetic acid (EDTA), 222	Flatus, 246	comparison to Clinitest tablet, 64
EU. See Ehrlich units	Fluorescence in situ hybridization (FISH), 237	Glucose tolerance test (GTT), 35
Excretion	Fluorescence lifetime, 91	Glucosuria, 62
secreted hydrogen ions with ammonia, 18f	Fluorescence microscopy, 91–92	Glutamine, in cerebrospinal fluid, 192
secreted hydrogen ions with phosphate, 18f	Fluorescent mapping spectral karyotyping (SKY),	Glycogenesis, 62
Exogenous procedure, 19	237	Glycogenolysis, 62
Explosive hazards, 8	Fluorescent microscopy, 92f	Glycosuria, 62
External quality control, 127	Fluorescent-treated antibody-absorption (FTA-	Goodpasture syndrome, 144–145 Gown, 3
Exudates, 223 laboratory differentiation from transudates,	ABS), 194 Foam shake test, 241	Gowii, 5 Gram stains, 85
223t	Foam stability index, 241	,
Eye protection, 3	FOBT. See Fecal occult blood testing	cerebrospinal fluid, and, 193–194 Granular casts, 108–109, 108f, 110f
Lyc protection, 5	Focal segmental glomerulosclerosis (FSGS), 146	Greiss reaction, 72
F	Følling, Ivan, 161	Guthrie test, 162f
Face shield, 3	Food and Drug Administration (FDA), 131, 136,	Gutilile test, 1021
FAH. See Fumarylacetoacetate hydrolase	181	Н
Fanconi syndrome, 58, 149, 165, 167	Froin syndrome, 180	Handwashing, 3–5, 5f
Fatty casts, 107, 107f, 108f	Fructose screening test, 170	Hansel stains, 85
FDA. See Food and Drug Administration	Fructosuria, 170	Harmonic oscillation densitometry (HOD), 46, 48
Fecal analysis, 245–254	Fumarylacetoacetate hydrolase (FAH), 163	Hartnup disease, 149, 165
Fecal carbohydrates, 253–254	i umarytacetoacetate mydrotase (17111), 105	Hazardous materials symbols, 7f
Fecal enzymes, 253	G	Hazards
Fecal fat, 251–252	Galactose, 64	biological, 2–5
analysis, 252t	Galactose-1-phosphate uridyl transferase (GALT),	chemical, 6–7
Fecal leukocytes, 249	170	electrical, 7–8
methylene blue stain procedure for, 249	Galactosemia, 64	physical, 8
Fecal material, 120f	Galactosuria, 64, 170	radioactive, 7
Fecal occult blood testing (FOBT), 250, 251	Gardnerella vaginalis, 97	sharp, 5–6
Fecal osmotic gap, 247	Gastrocolic fistulas, 249	Hematocrit, 224
Fecal screening tests, summary of, 254t	Gastrointestinal tract, fluid regulation in, 246f	Hematuria, 65, 66
Feces	Gault, H. H., 21	Heme, 167, 168f
chemical testing of, 250–252	Gestational diabetes, 62	Hemocytometer, 263, 265
macroscopic screening of, 248–249	Glans, 36	Hemoglobin, 105
appearance, 248–249	Glans penis, 200f	degradation, 68f
color, 248	Glomerular disorders, 144–145	Hemoglobinuria, 65–67
microscopic examination of, 249–250	clinical information associated with, 148t	Hemolytic disease of the newborn (HDN), 237,
fecal leukocytes, 249	laboratory testing for, 146–147t	238
muscle fibers, 249, 250	Glomerular filtrate, 17f	Hemoptysis, 145
, ,	·	A 2

Hemosiderin, 66	J	Lymphatic capillaries, 222
Hemosiderin granules, 99f	Jaundice, 68t	Lymphoblasts, 188f
Hemothorax, 224	Joint cavity, 212f	Lymphocytes, 184, 265, 265f
Henoch-Schönlein purpura, 145	Joint Commission on Accreditation of Healthcare	normal, 184f
Hepatitis B virus (HBV), 2, 4	Organizations (JCAHO), 8, 131, 139	viral meningitis and, 186f
HIAA. See 5-Hydroxyindoleacetic acid	Joint disorders	Lymphoma, 188
Hippocrates, 30	classification and pathologic significance of,	Lymphoma cells, 189f
Hippuric acid, 32t	212t	3.6
Histoplasma capsulatum, 265	laboratory findings in, 212t	M
Hoesch reagent, 71	Juxtaglomerular apparatus, 13f, 14, 15f	Macrophages, 184, 186f, 187f, 265, 265f
Hoesch test, 71–72, 168	Juxtaglomerular cells, 14, 15f	Macroscopic screening, urine, 82, 82t
Homocystinuria, 167	Juxtamedullary nephrons, 12, 13	Macula densa, 14, 15f
Homogentisic acid test, 163	**	Magnesium, 32t
Human chorionic gonadotropin (hCG), 259	K	Malabsorption syndrome, 165, 245–248
Human immunodeficiency virus (HIV), 2, 191,	Ketones	Maldigestion syndrome, 245–248
201	clinical significance of in urine, 64–65	Male genitalia, 200f
lymphocytes and, 184 Hunter syndrome, 169	reaction interference in urine, 65	Malignant cells, characteristics, 228t
	reagent strip reaction to in urine, 65	hematological origin, 188
Hurler syndrome, 169	summary of reagent strip, 66	nonhematological origin, 188–189
Hyaline casts, 90, 102–104, 104f Hyaluronic acid, 211	in urine, 64–65	Malignant melanoma, 163
	Ketonuria, 64	Maple syrup urine disease, 164–165 MAR. See Mixed agglutination reaction test
Hydrostatic pressure, 13, 14f Hyperglycemia, 62	Kidney stones. See Renal calculi	
Hypernatremia, 23	Klebsiella (bacteria), 44	Mask, 3 Mass gravity meter, 48f
Hypersthenuric, 48	Kleihauer-Betke test, 238	Material Safety Data Sheets (MSDS), 7
Hypoglycemia, 247	Köhler illumination, 89, 89f	Maximal reabsorptive capacity, 16
Hyponatremia, 23	KOVA stain, 85	MBP. See Myelin basic protein
Hyposthenuric, 48	Kruger's strict criteria, 204	MDRD. See Mychil Basic protein MDRD. See Modification of Diet in Renal Disease
Hypothalamus, 149	L	Meconium, 238
Trypothalamus, 179	Labia, 36	Medical errors, 139
I	Laboratory, clinical, safety in, 1–8	Medicare End Stage Renal Disease Program, 21
Iatrogenic compounds, 110	Laboratory information system (LIS), 259	Medulla, 13f
IBS. See Irritable bowel syndrome	Lactate, in cerebrospinal fluid, 192	Medullary interstitium, 13
Ictotest procedure, 69	Lactic dehydrogenase (LD) ratios, 228–229	Medulloblastoma, 189f
Ictotest tablets, for bilirubin in urine, 68–69	Lactosuria, 170	Melanoma, 44
Ideal body weight (IBW), 21	Lamellar body density, 240	Melanuria, 163
Idiopathic lactase deficiency, 253	Latex agglutination and enzyme-linked	Melituria, 170
Immune complexes, 144	immunosorbent assay (ELISA), 193	Membranoproliferative glomerulonephritis
Immunobead test, 206, 207	Lecithin-sphingomyelin (LS) ratio, 240	(MPGN), 145
Immunochemical fecal occult blood test (iFOBT),	Legionella pneumophila, 265	Membranous glomerulonephritis, 145
251	Lesch-Nyhan syndrome, 113	Meningitis, 180
Immunodip, 60	Leucine crystals, 118f	laboratory results, major, 192t
Immunofixation electrophoresis (IFE), 191	Leukocyte, 180. See also White blood cells	Mercaptoethane sulfonate sodium (MESNA), 65
Immunofluorescence, 91	Leukocyte esterase	Mesothelial cell, 221, 225
Immunoglobulin A (IgA), 144	clinical significance of in urine, 73–74	normal, 226f
Immunoglobulin A nephropathy, 145	clinical significance summary, 74	normal and reactive, 226f
Immunoglobulin G antibodies (IgG), 190-191,	reaction interference in urine, 74	reactive, 226f
206	reagent strip reaction to in urine, 74	Metabolic acidosis, 18
Immunoglobulin G (IgG) index, 191	reagent strip summary, 74	Metabolic and tubular disorders
In vitro fertilization (IVF), 199, 207	in urine, 73–74	clinical information associated with, 150t
Inborn error of metabolism (IEM), 160, 164, 166	Leukocyte esterase (LE) test, 73, 74	laboratory testing for, 150t
Indicanuria, 165–166	Leukocyturia, 74	Metabolic disorders, urine screening for,
Infertility, 201–202	Levy-Jennings control chart, 132, 133f, 262	160–171
Influenza A and B viruses, 265	Light-emitting diode (LED), 259	Micral test, 60
Institute of Medicine (IOM), 139	Liley graph, 240f	Microalbuminuria, 58
Interference-contrast microscopy, 90–91	Linen, precautions with, 3	testing for, 60–61
Internal quality control, 127	Lipid stains, 85	Microscope, 87–89
monitoring, 133	Lipiduria, 116	care of, 87t
Interstitial disorders, 149–150	Lipophages, 231f	parts of, 88f
clinical information associated with, 152t	Lithiasis, 152	resolution, 88
laboratory results in, 151t	Lithotripsy, 152	Microscopy
Inulin clearance, 19	Loops of Henle, 12–13, 15–17, 103	polarizing, 90
iQ200 Automated Urinalysis System, 260t	thick ascending, 13f	types of, 89–92
iQ200 Automated Urine Microscopy Analyzer,	thick descending, 13f	bright-field, 89
260t, 262	thin ascending, 13f	dark field, 91
Iris Diagnostics, 260t, 262	thin descending, 13f	differential interference-contrast, 91f
Irritable bowel syndrome (IBS), 247	LS ratio. See Lecithin–sphingomyelin ratio	fluorescence, 91–92
Isoelectric focusing (IEF), 191	Lupus erythematosus, 218	interference-contrast, 90–91
Isosthenuric, 48	Lyme disease, 218	phase-contrast, 89–90

289

Microviscosity:fluorescence polarization assay, 241	Nuclear irregularities, enhancement of, 227f Nucleated red blood cell (NRBC), 184, 185f	Peritoneal lavage, 229 Peritonitis, 229
Miditron Junior II, 260t, 261		Peritubular capillaries, 13, 13f, 17f
Milliosmole (mOsm), 22–23	O	Personal protective equipment (PPE), 2, 4
Minimal change disease, 146	Occult blood, 248, 250–251	рН
Mixed agglutination reaction (MAR) test, 206	Occupational Exposure to Blood-Borne Pathogens	clinical significance of in urine, 56–57
Mixed cellular casts, 90	Standard, 4	reagent strip reaction in urine, 56–57
Modification of Diet in Renal Disease (MDRD), 21	Occupational health and blood-borne pathogens,	summary of reagent strip, 57
Monoblasts, 188f	3–4	of urine, 56–57
Monocytes, 184	Occupational Safety and Health Administration	Phagocytic cells, 68
normal, 184f	(OSHA), 3, 6	Phase-contrast microscopy, 89–90, 90f
viral meningitis and, 186f	Oligoclonal bands, 191	Phase microscopy, 207
Monosodium urate (MSU), 214–216	Oligohydramnios, 236	Phenazopyridine, 43
crystals, 217f	Oliguria, 31	Phenolsulfonphthalein (PSP), 24
crystals, unstained, 215f	Oncotic pressure, 13–14, 14f, 221–222	Phenylalanine hydroxylase, 161
extracellular crystals, 216f	Organic acidemias, 165	Phenylalanine, metabolism of, 162f
strongly birefringent, 216f	Orthostatic proteinuria, 34, 58	Phenylketonuria, 161–163
Mosenthal concentration test, 22	OSHA Federal Hazard Communication Standard,	Phenylpyruvate, 161
Movable joints, See Diarthroses	7	Phenylpyruvic acid, 162–163
MPGN. See Membranoproliferative	Osmolar clearance, 24	Phosphate, 32t
glomerulonephritis	Osmolarity, 22–23	Physical hazards, 8
MS/MS. See Tandem mass spectrophotometry	clinical significance of, 23	Physiology, of semen, 199-200
Mucin clot test, 213. See also Ropes clot test	free water clearance, 23-24	Pia mater, 178, 178f
Mucopolysaccharide disorders, 169-170	freezing point osmometers, 23	Pigmented villonodular synovitis, 214
Mucopolysaccharide (MPS) paper test, 169	technical factors, 23	pK. See Dissociation constant
Mucus, in urine sediment, 102	vapor pressure osmometers, 23	Placenta, 236f
Mucus threads, 102f	Osmole, 22	Plan-Do-Check-Act (PDCA), 137
Multiples of the median (MoM), 239	Osmotic diarrhea, 253	Plan-Do-Study-Act (PDSA), 137
Multistix, 54, 56, 67, 70	Osmotic gradient, 13	Pleocytosis, 183
Multistix PRO reagent strips, 61, 259	Out-of-control procedures, 133f	Pleural fluid, 223–228
Mycobacterium tuberculosis, 265	Oval fat body, 99–100, 99f	adenocarcinoma, 226f, 227f
Mycoplasma pneumoniae, 265	Ovarian carcinoma cells, 231f	algorithm for testing, 228f
Myelin basic protein (MBP), 191	Overflow type versus renal type disorders, 160	appearance, 225
Myeloblasts, 188f		chemistry tests on, 226-227
Myoglobinuria, 66	P	correlation with disease, 224t
	p-aminohippuric acid (PAH) test, 24	formation and absorption, 222f
N	p-Nitroaniline test, 166	hematology tests, 225-226
National Committee for Clinical Laboratory	Pancreatic insufficiency, 249	LE cell in, 225f
Standards (NCCLS), 46	Paracentesis, 222	metastatic breast carcinoma cells in, 227f
National Fire Protection Association (NFPA),	Parasites, 100	microbiologic and serologic tests, 227-228
6, 8	Pareto chart, 138f	normal mesothelial cells, 225f
Near-infrared reflectance spectroscopy (NIRA),	Parietal membrane, 221	plasma cells, 226f
252	Passive transport, 15	significance of cells seen in, 225t
Negative birefringence, 216	Pasteur pipette, 181	significance of chemical testing of, 229t
Nephrogenic diabetes insipidus, 149	Patient care equipment, 3	small-cell carcinoma, 227f
Nephrons, 12	Patient placement, 4	PM. See Preventive maintenance
component parts and, 13f	PDCA. See Plan-Do-Check-Act	Pneumocystis carinii, 265, 266, 266f
cortical, 12	PDMAI, 139	Pneumonia, 227
juxtamedullary, 12, 13	PDSA. See Plan–Do-Study-Act	Podocyte, 13, 14f
movement of substances in, 17f	Penis, 200f	Polarized light, diagram of, 90f
relationship to kidney and excretory system,	Pentosuria, 170	Polarizing microscopy, 90
12f	PEP. See Postexposure prophylaxis	Pollen grain, 120f
relationship to renal function tests, 19f	Pericardial fluid, 228–229	Polydipsia, 31
Nephrotic syndrome, 116, 144-146	appearance, 228	Polyhydramnios, 236
Neubauer counting chamber, 181, 181f,	laboratory tests, 228-229	Polyuria, 31
202–203, 202f, 213, 223	malignant effusion, 229f	Porphobilinogen, 44, 70–71
Neural tube defects, 239	significance of testing, 229t	Hoesch screening test for, 71-72
Neutrophils, 184, 185f, 265	Pericardiocentesis, 222	Porphyrias, 167
Newborn screening tests, 160-161	Pericarditis, 228	summary of most common, 169t
1996 Comprehensive Accreditation Manual for	Peripheral blood (PB), 184	Porphyrin disorders, 167-168
Hospitals (JCAHO), 139	Peritoneal fluid, 229-232	Porphyrins, 44
Nitrite	appearance, 230	Porphyrinuria, 167
clinical significance of in urine, 72	laboratory tests, 230–232	Positive patient outcomes, 137
reaction interference in urine, 73	cellular examination, 230	Postexposure prophylaxis (PEP), 4
reagent strip reaction to in urine, 72-73	chemical testing, 230-232	Postrenal proteinuria, 58
reagent strip summary, 73	microbiology tests, 232	Potassium, 32t
in urine, 72–73	significance of testing, 230t	PPE. See Personal protective equipment
Nitroso-naphthol test, 163	transudates versus exudates, 229-230	Pre- and postmassage test (PPMT), 37
Nocturia, 31	yeast in, 231f	Prerenal proteinuria, 57-58

Preventive maintenance (PM), 129 Renal blood flow, 12-13 production, 201 Procedure manual, urinalysis, 128-135 specimen collection, 200-201 Renal calculi, 152 Procedure review documentation, 128f Renal concentration, 16f specimen handling, 201 Procedures, out-of-control, 133f effects of hydration on, 22 Semen analysis, 201–208 Renal disease, 143-153 Proficiency testing (PT), 127, 133 additional testing, 205-208 Prostate, 199, 200f glomerular disorders, 144-145 antisperm antibodies, 206-207 adenocarcinoma of, 231f Renal failure, 151-152 appearance, 201 chemical testing, 207 Protein, clinical significance of in urine, 57, 59 causes of acute, 152t Protein error of indicators, 58 Renal function, 11-25, 18-25 liquefaction, 201 Proteinuria, 57 glomerular filtration tests, 18-22 microbial testing, 207 Proteinuria, postrenal, 58 tubular reabsorption tests, 22-24 normal chemical values, 207t Providencia (bacteria), 44 tubular secretion and renal blood flow tests, normal values for, 201t Provider-performed microscopy (PPM), 135 pH, 202 category, 136t Renal glycosuria, 149 postvasectomy, 207 Proximal convoluted tubule, 13, 13f, 14f, 97-98 Renal lithiasis, 152-153 quality control, 207-208 Prussian blue stains, 85-87 Renal physiology, 12-18 round cells, calculation of, 204-205 glomerular filtration, 13-14 seminal fluid fructose, 206 Psammoma bodies, 230 renal blood flow, 12-13 Pseudochylous material, 224 sperm concentration count, 202-203 Pseudomonas, 44 tubular reabsorption, 14-17 calculation of, 202-203 PSP. See Phenolsulfonphthalein tubular secretion, 17-18 sperm count calculation of, 202-203 Purine disorders, 170 Renal plasma flow, 13 Pyelonephritis, 72, 105-106, 108 Renal proteinuria, 58 sperm function tests, 207 glomerular proteinuria, 58 Pyuria, 95 sperm morphology, 203-205 microalbuminuria, 58 sperm motility, 203 tubular proteinuria, 58 sperm visibility, 205-206 Quality assessment (QA), 127-128 testing for abnormal, 206t Renal threshold, 16 Quality control (QC), 127-128, 131, 133 Renal tubular acidosis, 18, 25 viscosity, 202 sample instrument recording sheet, 132f Renal tubular cells, 15 volume, 201-202 Quality improvement follow-up report, 130f Renal tubular epithelial (RTE) cells, 95, 97-99, Seminal fructose screening test, 206 98f, 102, 105-106, 108, 146, 149, 152 Quality management, in urinalysis laboratory, Seminal vesicles, 200, 200f 137-139 casts, 107f Seminal vessels, 199 Quality system, 127 clinical significance of in urine, 98-99 Seminiferous tubules, 200 fragments of, 98f Sentinel Event Policies and Procedures, 139 R Renal type disorders, versus overflow type Serous fluid, 221-232 RAAS. See Renin-angiotensin-aldosterone system disorders, 160 exudates, 223 Radioactive hazards, 7 formation of, 221-222 Renal vein, 13 Radiographic dye crystals, 116-118 Renin, 14 general laboratory procedures, 223 Radioisotopes, 7 Renin-angiotensin-aldosterone system (RAAS), pericardial fluid, 228-229 Rapid gastric emptying (RGE), 247 13 - 14peritoneal fluid, 229-232 Rapid plasma reagin (RPR), 195 actions of, 16t pleural fluid, 223-228 specimen collection and handling, 222-223 Rapidly progressive (crescentic) algorithm of, 15f glomerulonephritis (RPGN), 144 Respiratory distress syndrome (RDS), 239 transudates, 223 RDS. See Respiratory distress syndrome Respiratory syncytial virus, 266 Serous membrane, 221 Reticuloendothelial system, 68 Reagent strip reactions, 61 Sertoli cells, 200 Reagent strips, 54-56 Reye syndrome, 192 Serum-ascites albumin gradient (SAAG), 230 RGE. See Rapid gastric emptying handling and storage of, 55 Sharp hazards, 5-6 quality control of, 55-56 Rh antibodies, crossing the placenta, 239f Siemens Medical Solutions Diagnostics, 259, reaction interference in urine, 59-61 Rhabdomyolysis, 66 260t, 263 summary of protein in urine, 59 Rheumatoid arthritis, 218 Silver nitroprusside test, 167 Six Sigma Quality Management, 139 technique, 54-55 Rheumatoid factor (RF), 228 testing summary, 55 Roche Diagnostics, 260t, 261, 263 Sjögren syndrome, 145 Rectum, 200f Ropes clot test, 213. See also Mucin clot test SKY. See Fluorescent mapping spectral Red blood cells (RBC), 44, 92-94, 94f, 120, 179, RPGN. See Rapidly progressive (crescentic) karyotyping SLE. See Systemic lupus erythematosus 213, 223 glomerulonephritis bronchoalveolar lavage and, 265 RPR. See Rapid plasma reagin Sodium chloride, 32t casts, 104-105, 105f Specific gravity crenated, 92f clinical significance of in urine, 74 dysmorphic, 93 SAAG. See Serum-ascites albumin gradient reaction interference in urine, 75 dysmorphic, 93f Safety hazards, 2t reagent strip reaction to in urine, 75, 75f reagent strip summary, 75 normal, 92f Sanfilippo syndrome, 169 summary of microscopic, 94 Schistosoma haematobium, 100 of urine, 74-75 urinalysis and, 262, 263 Scrotum, 200f Specimen handling, urine, 33-34 Refractometer, 47-48 SD. See Standard deviation integrity of, 33 calibration of, 48f Secondary syphilis, 145 policy for mislabeled, 129t steps in use of, 47f Sedi-Stain, 85 preservation of, 33-34 Reiter cells, 214 Semen, 199-208 Spectrophotometric bilirubin scan, 239f Relative centrifugal force (RCF), 83 composition, 200t Renal artery, 12 physiology of, 199-200 abnormalities in heads and tails, 205f

function tests, 207t

morphology, 203-205 motility, 203

motility, grading, 203t

Spermatozoa, 100-102, 102f

amorphous head, 204f

Spermatozoa structure, 204f

SSA. See Sulfosalicylic acid

Stains, urine sediment, 85-87

Prussian blue stains, 85-87

urine collection, 37

Standard deviation (SD), 132

environmental control, 3

pathogens, 3-4

patient care equipment, 3

patient placement, 4

Starch granules, 120f

Stercobilinogen, 69

Steatorrhea, 248

Stool, 246

eye protection, 3

handwashing, 3

face shield, 3

gloves, 3

gown, 3

linen, 3

mask, 3

Standard precautions, 3, 32, 201

Spindle-shaped cells, cluster, 188f

Squamous epithelial cells, 93f, 96-97, 96f

Stamey-Mears four-glass localized method, of

occupational health and blood-borne

Hazards of Materials (NFPA), 6

macroscopic characteristics, 249t

Strongyloides stercoralis, 265

Sulfosalicylic acid (SSA), 59

reporting turbidity, 60t

Suprapubic aspiration, urine of, 36

cells and inclusions seen in, 214t

crystal identification, 214-217

crystal types, 214-215

characteristics of, 215t

differential count, 213-214

crystal polarization, 216-217

slide preparation, 215-216

Subarachnoid space, 178f

Sudan III staining, 224

Sulfa crystals, 119f

Synovial cavity, 212f

cell counts, 213

Synovial fluid, 211-218

chemistry tests on, 217

color and clarity, 213

Sulfate, 32t

Standard System for the Identification of the Fire

Sternheimer-Malbin stain, 85, 94, 103-104, 107

Sulfosalicylic acid (SSA) precipitation test, 59-60

abnormalities, 205f

double head, 204f

double tail, 204f

immature, 205f

nonviable, 206f

Spinal cord, 178f

gram stains, 85

lipid stains, 85

Hansel stains, 85

Spermatids, 201

Index 291 microbiologic tests on, 217-218 collection procedure, 137f normal values for, 212t history and importance of, 29-30 physiology of, 211-212 microscopic techniques, 87t

serologic tests on, 218 routine correlations, 84t specimen collection and handling, 212-213 viscosity, 213 instruments for, 260t Synovial joints, 211, 212f Urinalysis laboratory Synovial membrane, 212f Synoviocytes, 211

Sysmex America, 262, 263 Sysmex UF-50 Urine Cell Analyzer, 262 regulatory issues, 135-136 Urinalysis procedure manual, 128-135 Sysmex UF-100 Urine Cell Analyzer, 260, 260t,

262, 263, 263f Systemic lupus erythematosus (SLE), 144

Tamm-Horsfall protein, 102, 103, 106 Tandem mass spectrophotometry (MS/MS), Testis, 200f Thoracentesis, 222

Three-glass collection, urine of, 36 Thrombosis, 145

Titratable acidity, 25

Total quality management (TQM), 137

Toxoplasma gondii, 230, 265 Transitional epithelial cells, 97 Transudates, 223, 223t Treponema pallidum, 91 Trichomonas, 74, 90 Trichomonas vaginalis, 100

Triple phosphate crystals, 115f, 116f

in urine, 115, 115f

Tryptophan disorders, 165-166

5-Hydroxyindoleacetic acid (HIAA), 166

indicanuria, 165-166 Tryptophan metabolism, 165f Tubular disorders, 147-149

Tubular proteinuria, 58 Tubular reabsorption, 14-17, 16t

collecting duct concentration, 17

mechanisms, 15-17 tests, 22-24

Tubular secretion and renal blood flow tests, 24 - 25

p-aminohippuric acid (PAH) test, 24 titratable acidity and urinary ammonia, 24-25

Tubule, 17f

Tubulointerstitial disease, 149

2,4-dinitrophenylhydrazine test (DPNH), 165

Tyrosine crystals, 118f

Tyrosine, metabolism of, 162f

Tyrosyluria, 163

U

Umbilical cord. 236f Unconjugated bilirubin, 238 Universal Precautions (UP), 3 Urate crystals, abnormal, 116-121 Urea, 32t Ureter, 200f Urethra, 200f Uric acid, 32t, 215

crystals, 112, 114f See also Monosodium urate

Urinalysis, 29-38

abnormal conditions detected in routine, 160t cause-and-effect diagram for, 138f

Urinalysis automation, 259-263 quality assurance and management in, 127-139 quality management in, 137-139 analytical factors, 129-134 external quality control monitoring, 131-133 instrumentation and equipment, 131 internal quality control, 133 personnel and facilities, 133-134 postanalytical factors, 134-135 results interpretation, 134-135 results reporting, 134 preanalytical factors, 129 quality control, 131 testing procedure, 131 Urinary bladder, 200f Urinary constituents, abnormal, disorders associated with, 161t Urinary crystals, 110-121 formation, 110 identification techniques, 110-112 normal, 112-115 characteristics of, 113t Urinary meatus, 36 Urinary screening tests, 170–171t Urinary tract infection (UTI), 72, 73, 95, 100, 149, 150 Urine 24-hour (timed) specimen, 36 procedure, 36 abnormal metabolic substances in, 160

2-hour postprandial specimen of, 35 24-hour (timed) specimen collection

acid and alkaline, causes of, 56t

bilirubin in, 68-69 blood in, 65-67

casts, summary of, 111-112 catheterized specimen, 36

changes in unpreserved, 33t chart for analysis of, 31f

chemical examination of, 53-75

clarity of, 44-45

color and clarity procedures, 44

color of, 42-44

abnormal, 43-44

laboratory correlation of, 42-43t normal, 42-43

composition, 31, 32t

disposal of sample and container, 6f drug specimen collection, 37-38

epithelial cells in, 95-100 examination by physician, 30f

fasting specimen of, 34 first morning specimen of, 34

formation, 31 glucose in, 61-64

glucose tolerance specimens of, 35 instruction in examination of, 30f

ketones in, 64-65

leukocyte esterase in, 73-74

macroscopic screening of, 82 microscopic examination of, 81–121 microscopic reporting format, 134f midstream clean-catch specimen, 36 nitrite in, 72–73 odor of, 49 pediatric specimen of, 37 pediatric specimen of, 37 pilysical examination of, 41–49 preservatives, 34–351 prostatitis specimen, 36–37 protien in, 57–61 random specimen of, 37 red blood cells, 92–94 yeast, 93 red blood cells, 98 examination of, 82–84 sediment content bacteria, 100 casts, 102–110 crystals, 110–121 micsellaneous structures, summary of, 103 mucus, 102 parasites, 100 spermatozoa, 100–102 yeast, 100 sediment stain characteristics, 85 specific gravity of, 45–49, 74–75 specimen collection, 32–33 specimen spantion and summer of, 36 specimen stain characteristics, 85 specific gravity of, 45–49, 74–75 specimen collection, 32–33 specimen and ling, 33–34 specimen specimen of, 68 urbola of the plenylalanine-tyrosine disorders of the phenylalanine-tyrosine pallway, 164 nacroscopic examination of, 82 nations (38–84) volume and examination of, 82 nations (38–84) value (38–49 value (38–49) value (48–49)	Urine (Continued)	metabolic disorders, for, 160–171	reagent strip reaction to in urine, 70
microscopic examination of, 81–121 midstream clean-carch specimen, 36 nitrite in, 72–73 cdor of, 49 pediatric specimen of, 37 physical examination of, 41–49 preservatives, 34–351 prostatitis specimen, 36–37 protein in, 57–61 random specimen of, 34 reagent strip reaction to protein in, 58–59 red color differentiation of testing positive for blood, 44f sediment content bacteria, 100 casts, 102–110 crystals, 110–121 miscellaneous structures, summary of, 103 mucus, 102 parasites, 100 speriment sain characteristics, 851 specimen stain characteristics, 851 specimen spiration, 33–34 specimen spiration, 33–34 specimen spiration, 33–34 specimen spiration, 33–34 specimen tonlent content content specimen of, 83 micus, 102 parasites, 100 spermatozoa, 100–102 parasites, 100 spermatozoa, 1			
microscopic reporting format, 134f midstream clean-catch specimen, 36 nitrite in, 72–73 odor of, 49 pediatric specimen of, 37 pH, 56–57 physical examination of, 41–49 preservatives, 34–351 prostatitis specimen, 36–37 protein in, 78–61 random specimen of, 34 reagent strip reaction to protein in, 58–59 red color differentiation of testing positive for blood. 44f sediment content bacteria, 100 casts, 102–110 crystals, 110–121 miscellaneous structures, summary of, 103 mucus, 102 parasites, 100 spermatozoa, 100–102 parasites, 100 spermatozoa, 100–102 yeast, 100 sediment stain characteristics, 851 specimen toplane, 33–34 specimen toplane, 33–34 specimen toplane, 33–34 speciment protein in, 53–39 pration of testing bositive for blood, 41 Urino structures, summary of, 103 mucus, 102 Urine specific gravity clinical correlations, 48–49 harmonic oscillation densitometry, 48 specific gravity of, 45–49, 74–75 specimen collection, 32–33 specimen types, 34–38, 351 suprapubic aspiration of, 36 volume, 31–32 Urine clarity, 45 normal, 44 turbidity, nonpathologic, 44–45 Urinos graveling of, 45–68, 70 disorders of the phenylalanine-tyrosine constituents, 92–121 constituents, 92–121 cespected staining reactions, 861 red blood cells, 92–94 vasa, 93 red blood cells, 92–94 vasa, 93 vase, 93 vase tect, 13, 13f vasectomy reversal, 206 vase tecta, 1, 105 vasectomy reversal, 206 vase tecta, 1, 105 vase tecta, 1, 106 vase, 88–82+4 veneral Disease Research Laboratory (VDRL), 194–195 visceral membrane, 221 vesicoureteral reflux, 150 veneral Disease Research Laboratory (VDRL), 194–195 visceral membrane, 221 vesicoureteral reflux, 150 veneral Disease Research Laboratory (VDRL), 194–195 visceral membrane, 221 vesicoureteral reflux, 150 vase tecta, 106 vase tecta,			
midstream clean-catch specimen, 36 nitrite in, 72–73 odor of, 49 pediatric specimen of, 37 ph, 56–57 ph, 56–57 physical examination of, 41–49 preservatives, 34–351 prostatitis specimen, 36–37 protein in, 57–61 random specimen of, 34 reagent strip reaction to protein in, 58–59 red color differentiation of testing positive for blood, 44f sediment content bacteria, 100 casts, 102–110 crystals, 110–121 miscellaneous structures, summary of, 103 mucus, 102 parasites, 100 sediment stain characteristics, 851 specimen taian characteristics, 851 specimen taian characteristics, 851 specimen and ing, 33–34 specimen ptps, 34–38, 351 surapubic aspiration of, 36 volume, 31–32 Urine clarity, 45t Urine carreining Urine screening Urine screen	*		,
nutrie in, 72–73 codor of, 49 pediatric specimen of, 37 pht, 56–57 physical examination of, 41–49 preservatives, 34–351 prostatitis specimen, 36–37 protein in, 57–61 random specimen of, 34 reagent strip reaction to protein in, 58–59 red color differentiation of testing positive for blood, 44f sediment content bacteria, 100 casts, 102–110 crystals, 110–121 miscellaneous structures, summary of, 103 mucus, 102 parasites, 100 spermatozoa, 100–102 parasites, 100 sediment stain characteristics, 85t specimen collection, 32–33 specimen handling, 33–34 specimen handling, 33–34 specimen handling, 33–34 specimen ptypes, 34–38, 35t volume, 31–32 Urine clarity, 45t Urine screening disorders of the phenylalanine-tyrosine red blonded cells, 92–94 reagent strip reaction to protein in, 58–59 red color preparation and examination of, 82–84 preparation of, 83 preparation of, 83 preparation of, 84 preparation of, 84 preparation of, 84 preparation of, 85 preparation of, 84 preparation of, 84 preparation of, 85 preparation of, 85 preparation of, 85 preparation of, 84 preparation of, 85 preparation of, 84 preparation of, 85 preparation of, 84 preparation of, 84 preparation of, 82–84 preparation of, 85 preparation of, 86 preparation of, 87 preparation of, 87 preparation of, 88 preparation of, 88 preparation of, 84 preparation			OTI. See Officially tract finection
odor of, 49 pediatric specimen of, 37 pht, 56–57 physical examination of, 41–49 preservatives, 34–35t prostatitis specimen, 36–37 protein in, 57–61 random specimen of, 34 reagent strip reaction to protein in, 58–59 red color differentiation of testing positive for blood, 44f sediment content bacteria, 100 casts, 102–110 crystals, 110–121 miscellaneous structures, summary of, 103 mucus, 102 parasites, 100 spermatozoa, 100–102 parasites, 100 spermatozoa, 100–102 parasites, 100 sediment stain characteristics, 85t specimen typea, 23–33 specimen typea, 24–95 Urine carries, 48 Urines yellogic, 45 suparpublic aspiration of, 36 volume, 31–32 Urine clarity, 45t normal, 44 turbidity, nonpathologic, 44–45 Urines zeroeining disorders of the phenylalanine-tyrosine exexeted statning reactions, 86t red blood cells, 92–94 van de Kamer titration, 251, 252 vasa correlation fresults, 84 vascasostomy. See vasectomy reversal vasorasostomy. See vasectomy reversal vasorasostomy. See vasectomy reversal. vasorasostomy. See vase			\mathbf{V}
pediatric specimen of, 37 pH, 56–57 yeast, 93 yeast, 93 yeast, 93 yeast, 100 casts, 102–110 spersmatozoa, 100–102 parasites, 100 sediment stain characteristics, 851 specimen stain characteristics, 851 specimen handling, 33–34 specimen and more and processing and processing specimen of the phenylalanine-tyrosine disorders of the phenylalanine-tyrosine disorders, 120 correlation of results, 84 yeast, 92 west, 100 content, 96 correlation of, 83–84 yeasting 67 correlation of, 83 recordent on protein in, 58–59 red color differentiation of testing positive for blood, 44f sediment content bacteria, 100 casts, 102–110 specimen volume, 83 yeast, 100 seadiment stain characteristics, 851 specimen stain characteristics, 851 suprapuble appraisation of, 82–84 pathologic causes of, 451 turinomer, 46, 46f urnoment, 44 turbidity, nonpathologic, 44–45 Urobilin, 69 Urobilin, 69 Urobilin, 69 (disorders of the phenylalanine-tyrosine) and the processing positive for blood cells, 92–94 yeast, 100 yeast parable averaged and stain faracteristics, 851 turinomer, 46, 46f urobilinogen, 54, 68, 70 clinical significance of in urine, 70 yeast parable and the same rituration, 251, 252 vas determs, 200f vasceterins, 13, 13f vasceterins, 200f vasceterins, 12, 126 vasceterins, 200f vascet		,	•
pH, 56–57 physical examination of, 41–49 preservatives, 34–351 prostatitis specimen, 36–37 protein in, 57–61 random specimen of, 34 reagent strip reaction to protein in, 58–59 red color differentiation of testing positive for blood, 44f sediment content bacteria, 100 casts, 102–110 crystals, 110–121 miscellaneous structures, summary of, 103 mucus, 102 parasites, 100 speriment ozone speriment ozone, 100–102 spersmatozoa, 100–102 spersmatozoa, 100–102 spersmatozoa, 100–102 spersmatozoa, 100–102 speriment protein for disting correlations of, 85 specimen toolicetion, 32–33 specimen tomostation of, 85 specimen toprotein in, 85 specimen to make the measurements of, 48 specimen to make the measurements of, 48 specimen to more samination of, 84 specimen to more than the measurements of, 48 specimen type specime		1 0	1 1 1 7
physical examination of, 41–49 preservatives, 34–35t prostatitis specimen, 36–37 protein in, 57–61 random specimen of, 34 reagent strip reaction to protein in, 58–59 red color differentiation of testing positive for blood, 44f sediment content bacteria, 100 casts, 102–110 crystals, 110–121 miscellaneous structures, summary of, 103 mucus, 102 parasites, 100 spermatozoa, 100–102 parasites, 100 sediment stain characteristics, 85t specimen rollection, 32–33 specimen rollection, 32–33 specimen rollection, 32–33 specimen spiration of, 36 volume, 31–32 Urine clarity, 45t normal, 44 Urisys 2400 system, 260t, 261 Urine streening disorders of the phenylalanine-tyrosine content, 96f correlation of results, 84 correlation of results, 84 vasination of, 83-84 vascoms reversal, 206 Vascoreversal, 206 Vascoreversal 206 Vascoreve			
preservatives, 34–35t prostatitis specimen, 36–37 protein in, 57–61 random specimen of, 34 reagent strip reaction to protein in, 58–59 red color differentiation of testing positive for blood, 44f sediment content bacteria, 100 casts, 102–110 crystals, 110–121 miscellaneous structures, summary of, 103 mucus, 102 parasites, 100 spermatozoa, 100–102 parasites, 100 sediment stain characteristics, 85t specimen rollection, 32–33 specimen tollection, 32–33 specimen landling, 33–34 specimen collection, 32–33 specimen landling, 33–34 specimen landling, 33–35 suprapublic aspiration of, 36 Vascotomy reversal, 206 vasamination of, 83–84 vasorysostomy, See Vasectomy reversal Vasorysostomy, See Vasory tasory ovasory (VDRL), 194–195 vesicourcteral Disease Research Laboratory (VDRL), 194–195 viscend membrane, 221 vesicourcteral reflux, 150 Watson-Schwatz differentiation test, 71, 167 interpretation of, 84 vasorysostomy, See Vasectomy reversal Venereal Disease Research Laboratory (VDRL), 194–195 viscend membrane	1 '		•
prostatitis specimen, 36–37 protein in, 57–61 protein in, 58–59 protein in, 58–58 pr			
protein in, 57–61 random specimen of, 34 reagent strip reaction to protein in, 58–59 red color differentiation of testing positive for blood, 44f sediment content bacteria, 100 casts, 102–110 crystals, 110–121 miscellaneous structures, summary of, 103 mucus, 102 parasites, 100 speriment stain characteristics, 85t yeast, 100 sediment stain characteristics, 85t specimen toil collection, 32–33 specimen toil collection, 32–33 specimen type, 34–38, 351 specimen type, 34–38, 351 suprapublic aspiration of, 36 volume, 31–32 Urine clarity, 45t normal, 44f turbidity, nonpathologic, 44–45 Urine sunface and individed and individed and incompleted and incompleted and incompleted and incompleted as incomplete and incompleted as incompleted as incomplete, as collinical significance of in urine, 70 valous examination of, 82–84 viscoral membrane, 221 vesicoureteral reflux, 150 vesicoureteral reflux, 150 We seciment of, 84 vesicoureteral reflux, 150 We seciment preparation, 83 was permation, 83 was permation of, 84 vesicoureteral reflux, 150 We seciment preparation of, 84 vesicoureteral reflux, 150 We ston-Schwartz differentiation test, 71, 167 interpretation of, 72t White blood cells (vest, 94, 94, 95, 94) to hi	¥ .	· · · · · · · · · · · · · · · · · · ·	
random specimen of, 34 reagent strip reaction to protein in, 58–59 red color differentiation of testing positive for blood, 44f sediment content bacteria, 100 casts, 102–110 crystals, 110–121 miscellaneous structures, summary of, 103 mucus, 102 parasites, 100 spermatozoa, 100–102 parasites, 100 sediment atian characteristics, 85t sperimen collection, 32–33 specimen types, 34–38, 35t suprapubic aspiration of, 36 Volume, 31–32 Urine claricy, 45 normal, 44 turbiidity, nonpathologic, 44–45 Urine searing disorders of the phenylalanine-tyrosine read blood cells, 90 microscopic examination of, 82–84 preparation of, 83 red blood cells, 93–94 read blood cells, 93–94 visceral membrane, 221 Venereal Disease Research Laboratory (VDRL), 194–195 Visceral membrane, 221 Vesicoureteral reflux, 150 Weston-Schwartz differentiation test, 71, 167 interpretation of, 72t waxy casts, 109, 109f, 110f Watson-Schwartz differentiation test, 71, 167 interpretation of, 72t Waxy casts, 109, 109f, 110f Wegner's granulomatosis, 145 Wegner's granulomatosis, 145 White blood cell count, 201 White blood cell count, 201 White blood cell count, 201 White blood cells (WBC), 45, 94f, 95f, 100, 100f, 107, 179, 223 bronchoalveolar lavage and, 265 casts, 105–106, 106f clump of, 94f summary of microscopic, 95f urinalysis and, 262, 263 World Health Organization (WHO), 203–204, 207 Suprapubic aspiration of, 36 Volume, 31–32 Viries claricy, 45t Vir			
reagent strip reaction to protein in, 58–59 red color differentiation of testing positive for blood, 44f reporting microscopic examination of, 82–84 bacteria, 100 casts, 102–110 crystals, 110–121 miscellaneous structures, summary of, 103 mucus, 102 parasites, 100 spermatozoa, 100–102 spermatozoa, 100–102 sperment stain characteristics, 85t sperminent stain characteristics, 85t specimen repath specific gravity of, 45–49, 74–75 specimen collection, 32–33 specimen handling, 33–34 specimen types, 34–38, 35t spermatozoa, 13–32 Urine clarity, 45t normal, 44 turbidity, nonpathologic, 44–45 Urine specific gravity of incompanient of, 82–84 viscoureteral reflux, 150 westion of, 28 vestion of, 28 was assimilation of, 84 was casmination of, 84 westion of, 72t waxy casts, 109, 109f, 110f wegner's granulomatosis, 145 white blood cell count, 201 white b	1	,	
red color differentiation of testing positive for blood, 44f red blood cells, 93–94 sediment content bacteria, 100 casts, 102–110 crystals, 110–121 miscellaneous structures, summary of, 103 mucus, 102 parasites, 100 spermatozoa, 100–102 spermatozoa, 100–102 spermatozoa, 100–102 sediment stain characteristics, 85t specifine gravity of, 45–49, 74–75 specimen collection, 32–33 specimen handling, 33–34 specimen types, 34–38, 35t suprapubic aspiration of, 36 Urinos districture, 46, 46f volume, 31–32 Urine clarity, 45t normal, 44 turbidity, nonpathologic, 44–45 Urine specifine gravity olionical correlation, 48, 46f Urobilin, 69 Clinical significance of in urine, 70 Viscoureteral reflux, 150 Vesicoureteral reflux, 150 W Watson-Schwartz differentiation test, 71, 167 interpretation of, 72 t Waxy casts, 109, 109f, 110f Wesper's granulomatosis, 145 White blood cells (WBC), 41 Wesper's granulomatosis, 145 White blood cells ount, 201 White blood cells (WBC), 45, 94f, 95f, 100, 100f, 107, 179, 223 bronchoalweolar lavage and, 265 casts, 105–106, 106f		•	
differentiation of testing positive for blood, 44f red blood cells, 93–94 www. Watson-Schwartz differentiation test, 71, 167 interpretation of, 72t wasy casts, 102–110 specimen volume, 83 interpretation of, 72t wasy casts, 109, 109f, 110f white blood cells, 94–95 white blood cells, 94–95 white blood cells, 94–95 white blood cells (WBC), 45, 94f, 95f, 100, 100f, parasites, 100 clinical correlations, 48–49 to spermatozoa, 100–102 harmonic oscillation densitometry, 48 perimen stain characteristics, 85t with the specific gravity clinical correlations in, 45t specimen collection, 32–33 nonpathologic causes of, 45t suprapubic aspiration of, 36 with collection, 45 harbologic causes of, 45t suprapubic aspiration of, 36 with collection, 94–95 wolume, 31–32 Urisys 1100 system, 260t, 261 volume, 31–32 Urisys 1100 system, 260t, 261 volume, 31–32 Urisys 1200 system, 260t, 261 volume, 31–32 Volume, 31–32 Urisys 1200 system, 260t, 261 volume, 31–32 Volume, 31–34 Volume, 31–34 Volume, 31–35 Volume, 31–35 Volume, 31–35 Volume, 31–35 Volume, 31–35 Volume, 31–35 Volu		* * * * * * * * * * * * * * * * * * * *	Visceral membrane, 221
sediment content red blood cells, 93–94 sediment content reporting microscopic examination of, 84 bacteria, 100 specimen preparation, 83 Watson-Schwartz differentiation test, 71, 167 casts, 102–110 specimen volume, 83 interpretation of, 72t crystals, 110–121 stains, 85–87 Waxy casts, 109, 109f, 110f miscellaneous structures, summary of, 103 white blood cells, 94–95 White blood cell count, 201 mucus, 102 Urine specific gravity White blood cells (WBC), 45, 94f, 95f, 100, 100f, 107, 179, 223 spermatozoa, 100–102 harmonic oscillation densitometry, 48 pronchoalveolar lavage and, 265 yeast, 100 measurements of, 48 casts, 105–106, 106f sediment stain characteristics, 85t Urine turbidity clump of, 94f specific gravity of, 45–49, 74–75 laboratory correlations in, 45t summary of microscopic, 95f specimen collection, 32–33 nonpathologic causes of, 45t urinalysis and, 262, 263 specimen types, 34–38, 35t pathologic causes of, 45t 207 suprapubic aspiration of, 36 Urinometer, 46, 46f Wright's stain, 95, 182 Urine clarity, 45t Urisys 1100 system, 260t, 261 Urisys 1100 system, 260t, 261 Urisys 2400 system, 260t, 261 Urisys 2400 system, 260t, 261 Urine screening Urobilinogen, 54, 68, 70 disorders of the phenylalanine-tyrosine clinical significance of in urine, 70 Watson-Schwartz differentiation test, 71, 167 interpretation of, 72t waxy casts, 109, 109f, 110f Waxy casts, 109, 109f, 110f Waxy casts, 109, 109f, 110f Wegner's granulomatosis, 145 Wegner's granulom			,
sediment content bacteria, 100 casts, 102–110 crystals, 110–121 stains, 85–87 waxy casts, 109, 109f, 110f miscellaneous structures, summary of, 103 mucus, 102 parasites, 100 speriment olume, 83 sperimen specific gravity sperimatozoa, 100–102	0.1	1 1	· · · · · · · · · · · · · · · · · · ·
bacteria, 100 casts, 102—110 casts, 102—110 crystals, 110—121 miscellaneous structures, summary of, 103 mucus, 102 parasites, 100 parasites, 100 specimen volume, 83 specimen volume, 83 white blood cells, 94—95 White blood cell count, 201 white blood cells, 94—95 White blood cells (WBC), 45, 94f, 95f, 100, 100f,	sediment content	,	W
casts, 102–110 crystals, 110–121 stains, 85–87 waxy casts, 109, 109f, 110f miscellaneous structures, summary of, 103 white blood cells, 94–95 White blood cell count, 201 mucus, 102 parasites, 100 spermatozoa, 100–102 yeast, 100 sediment stain characteristics, 85t specific gravity specific gravity of, 45–49, 74–75 specimen collection, 32–33 specimen types, 34–38, 35t suprapubic aspiration of, 36 volume, 31–32 Urine clarity, 45t normal, 44 turbidity, nonpathologic, 44–45 Urios yestem, 260t Urios linical cortelations, 48, 49 harmonic oscillation densitometry, 48 measurements of, 48 casts, 105–106, 106f clump of, 94f summary of microscopic, 95f urinalysis and, 262, 263 world Health Organization (WHO), 203–204, 207 Wright's stain, 95, 182 Vine clarity, 45t urrios 2400 system, 260t, 261 Urios ystem, 260t, 261 Urios ystem, 260t, 261 Urios gening Urobilinogen, 54, 68, 70 disorders of the phenylalanine-tyrosine interpretation of, 72t waxy casts, 109, 109f, 110f wegner's granulomatosis, 145 White blood cell count, 201 White blood cell sount, 201 White blood cells own, 201 white blood cells own, 201 white blood cells own, 201 white blood cell sount, 201 white blood cells own, 201 white blood	bacteria, 100		Watson-Schwartz differentiation test, 71, 167
miscellaneous structures, summary of, 103 white blood cells, 94–95 White blood cell count, 201 mucus, 102 Urine specific gravity White blood cells (WBC), 45, 94f, 95f, 100, 100f, parasites, 100 clinical correlations, 48–49 107, 179, 223 spermatozoa, 100–102 harmonic oscillation densitometry, 48 bronchoalveolar lavage and, 265 yeast, 100 measurements of, 48 casts, 105–106, 106f sediment stain characteristics, 85t Urine turbidity clump of, 94f specific gravity of, 45–49, 74–75 laboratory correlations in, 45t summary of microscopic, 95f specimen collection, 32–33 nonpathologic causes of, 45t urinalysis and, 262, 263 specimen handling, 33–34 pathologic, 45 pathologic causes of, 45t 207 suprapubic aspiration of, 36 Urinometer, 46, 46f Wright's stain, 95, 182 Urisys 1100 system, 260t, 261 Vrisys 1800 system, 260t, 261 X Urine clarity, 45t Urisys 1900 system, 260t, 261 X turbidity, nonpathologic, 44–45 Urobilin, 69 Xanthochromatic supernatant, 180 turbidity, nonpathologic, 44–45 Urobilin, 69 Xanthochromia, 179 Urobilinogen, 54, 68, 70 disorders of the phenylalanine-tyrosine clinical significance of in urine, 70 Y	casts, 102–110	specimen volume, 83	interpretation of, 72t
miscellaneous structures, summary of, 103 white blood cells, 94–95 White blood cell count, 201 mucus, 102 Urine specific gravity White blood cells (WBC), 45, 94f, 95f, 100, 100f, parasites, 100 clinical correlations, 48–49 107, 179, 223 spermatozoa, 100–102 harmonic oscillation densitometry, 48 bronchoalveolar lavage and, 265 yeast, 100 measurements of, 48 casts, 105–106, 106f sediment stain characteristics, 85t Urine turbidity clump of, 94f specific gravity of, 45–49, 74–75 laboratory correlations in, 45t summary of microscopic, 95f specimen collection, 32–33 nonpathologic causes of, 45t urinalysis and, 262, 263 specimen handling, 33–34 pathologic, 45 pathologic causes of, 45t 207 suprapubic aspiration of, 36 Urinometer, 46, 46f Wright's stain, 95, 182 volume, 31–32 Urisys 1100 system, 260t, 261 Vrisys 1800 system, 260t, 261 X turbidity, nonpathologic, 44–45 Urobilin, 69 Xanthochromatic supernatant, 180 turbidity, nonpathologic, 44–45 Urobilin, 69 Xanthochromia, 179 Urobilinogen, 54, 68, 70 disorders of the phenylalanine-tyrosine clinical significance of in urine, 70 Y		*	*
mucus, 102 parasites, 100 clinical correlations, 48–49 spermatozoa, 100–102 spermatozoa, 100–102 parasites, 100 sediment stain characteristics, 85t specimen collection, 22–33 specimen collection, 32–33 specimen handling, 33–34 specimen types, 34–38, 35t surpapubic aspiration of, 36 volume, 31–32 Urine yecific gravity Specimen collection, 32–33 specimen types, 34–38, 35t surpapubic aspiration of, 36 Urinometer, 46, 46f Volume, 31–32 Urine yetific gravity Urine yecific gravity Urine yecific gravity Urine yecific gravity Urine yecific gravity Specimen yecific gravity Specimen types, 34–38, 35t Specimen t	miscellaneous structures, summary of,	volume examined, 83	
parasites, 100 clinical correlations, 48–49 107, 179, 223 spermatozoa, 100–102 harmonic oscillation densitometry, 48 bronchoalveolar lavage and, 265 yeast, 100 measurements of, 48 casts, 105–106, 106f sediment stain characteristics, 85t Urine turbidity clump of, 94f specific gravity of, 45–49, 74–75 laboratory correlations in, 45t summary of microscopic, 95f specimen collection, 32–33 nonpathologic causes of, 45t urinalysis and, 262, 263 specimen handling, 33–34 pathologic, 45 World Health Organization (WHO), 203–204, specimen types, 34–38, 35t pathologic causes of, 45t 207 suprapubic aspiration of, 36 Urinometer, 46, 46f Wright's stain, 95, 182 urine stain, 45t Urisys 1100 system, 260t, 261 Urine clarity, 45t Urisys 2400 system, 260t, 261 Urine screening Urobilin, 69 Xanthochromatic supernatant, 180 Urobilinogen, 54, 68, 70 disorders of the phenylalanine-tyrosine clinical significance of in urine, 70 Vine screening clinical significance of in urine, 70 Vine speciment value bronchoalveolar lavage and, 265 bronchoalveolar lavage and, 265 casts, 105–106, 106f clump of, 94f summary of microscopic, 95f summary of micros	103	white blood cells, 94-95	White blood cell count, 201
parasites, 100 clinical correlations, 48–49 107, 179, 223 spermatozoa, 100–102 harmonic oscillation densitometry, 48 bronchoalveolar lavage and, 265 yeast, 100 measurements of, 48 casts, 105–106, 106f sediment stain characteristics, 85t Urine turbidity clump of, 94f specific gravity of, 45–49, 74–75 laboratory correlations in, 45t summary of microscopic, 95f specimen collection, 32–33 nonpathologic causes of, 45t urinalysis and, 262, 263 specimen handling, 33–34 pathologic, 45 World Health Organization (WHO), 203–204, specimen types, 34–38, 35t pathologic causes of, 45t 207 suprapubic aspiration of, 36 Urinometer, 46, 46f Wright's stain, 95, 182 urine stain, 45t Urisys 1100 system, 260t, 261 Urine clarity, 45t Urisys 2400 system, 260t, 261 Urine screening Urobilin, 69 Xanthochromatic supernatant, 180 Urobilinogen, 54, 68, 70 disorders of the phenylalanine-tyrosine clinical significance of in urine, 70 Vine screening clinical significance of in urine, 70 Vine speciment value bronchoalveolar lavage and, 265 bronchoalveolar lavage and, 265 casts, 105–106, 106f clump of, 94f summary of microscopic, 95f summary of micros	mucus, 102	Urine specific gravity	White blood cells (WBC), 45, 94f, 95f, 100, 100f,
yeast, 100 measurements of, 48 casts, 105–106, 106f sediment stain characteristics, 85t Urine turbidity clump of, 94f specific gravity of, 45–49, 74–75 laboratory correlations in, 45t summary of microscopic, 95f specimen collection, 32–33 nonpathologic causes of, 45t urinalysis and, 262, 263 specimen handling, 33–34 pathologic, 45 World Health Organization (WHO), 203–204, specimen types, 34–38, 35t pathologic causes of, 45t 207 suprapubic aspiration of, 36 Urinometer, 46, 46f Wright's stain, 95, 182 volume, 31–32 Urisys 1100 system, 260t, 261 Urine clarity, 45t Urisys 1800 system, 260t, 261 Urinomal, 44 Urisys 2400 system, 260t, 261–262 Xanthochromatic supernatant, 180 turbidity, nonpathologic, 44–45 Urobilin, 69 Xanthochromia, 179 Urine screening Urobilinogen, 54, 68, 70 disorders of the phenylalanine-tyrosine clinical significance of in urine, 70	parasites, 100	clinical correlations, 48-49	
sediment stain characteristics, 85t specific gravity of, 45–49, 74–75 laboratory correlations in, 45t specimen collection, 32–33 nonpathologic causes of, 45t specimen handling, 33–34 specimen types, 34–38, 35t specimen types, 34–38, 35t specimen types, 34–38, 35t suprapubic aspiration of, 36 Urinometer, 46, 46f Vright's stain, 95, 182 Volume, 31–32 Urisys 1100 system, 260t, 261 Urine clarity, 45t normal, 44 turbidity, nonpathologic, 44–45 Urobilin, 69 Urobilinogen, 54, 68, 70 disorders of the phenylalanine-tyrosine Urine turbidity supmany of microscopic, 95f supmany of microscopic pathologic, 45 supmany of microscopic pathologic patho	spermatozoa, 100–102	harmonic oscillation densitometry, 48	bronchoalveolar lavage and, 265
specific gravity of, 45–49, 74–75 laboratory correlations in, 45t summary of microscopic, 95f specimen collection, 32–33 nonpathologic causes of, 45t urinalysis and, 262, 263 specimen handling, 33–34 pathologic, 45 World Health Organization (WHO), 203–204, specimen types, 34–38, 35t pathologic causes of, 45t 207 suprapubic aspiration of, 36 Urinometer, 46, 46f Wright's stain, 95, 182 volume, 31–32 Urisys 1100 system, 260t, 261 Urine clarity, 45t Urisys 1800 system, 260t, 261 normal, 44 Urisys 2400 system, 260t, 261-262 Xanthochromatic supernatant, 180 turbidity, nonpathologic, 44–45 Urobilin, 69 Xanthochromia, 179 Urine screening Urobilinogen, 54, 68, 70 disorders of the phenylalanine-tyrosine clinical significance of in urine, 70	yeast, 100	measurements of, 48	casts, 105-106, 106f
specimen collection, 32–33 nonpathologic causes of, 45t urinalysis and, 262, 263 specimen handling, 33–34 pathologic, 45 World Health Organization (WHO), 203–204, specimen types, 34–38, 35t pathologic causes of, 45t 207 suprapubic aspiration of, 36 Urinometer, 46, 46f Wright's stain, 95, 182 volume, 31–32 Urisys 1100 system, 260t, 261 Urine clarity, 45t Urisys 1800 system, 260t, 261 normal, 44 Urisys 2400 system, 260t, 261-262 Xanthochromatic supernatant, 180 turbidity, nonpathologic, 44–45 Urobilin, 69 Xanthochromia, 179 Urine screening Urobilinogen, 54, 68, 70 disorders of the phenylalanine-tyrosine clinical significance of in urine, 70 Y	sediment stain characteristics, 85t	Urine turbidity	clump of, 94f
specimen handling, 33–34 pathologic, 45 World Health Organization (WHO), 203–204, specimen types, 34–38, 35t pathologic causes of, 45t 207 suprapubic aspiration of, 36 Urinometer, 46, 46f Wright's stain, 95, 182 volume, 31–32 Urisys 1100 system, 260t, 261 Urine clarity, 45t Urisys 1800 system, 260t, 261 normal, 44 Urisys 2400 system, 260t, 261–262 Xanthochromatic supernatant, 180 turbidity, nonpathologic, 44–45 Urobilin, 69 Xanthochromia, 179 Urine screening Urobilinogen, 54, 68, 70 disorders of the phenylalanine-tyrosine clinical significance of in urine, 70 Y	specific gravity of, 45-49, 74-75	laboratory correlations in, 45t	summary of microscopic, 95f
specimen types, 34–38, 35t pathologic causes of, 45t 207 suprapubic aspiration of, 36 Urinometer, 46, 46f Wright's stain, 95, 182 volume, 31–32 Urisys 1100 system, 260t, 261 Urine clarity, 45t Urisys 1800 system, 260t, 261 normal, 44 Urisys 2400 system, 260t, 261-262 Xanthochromatic supernatant, 180 turbidity, nonpathologic, 44–45 Urobilin, 69 Xanthochromia, 179 Urine screening Urobilinogen, 54, 68, 70 disorders of the phenylalanine-tyrosine clinical significance of in urine, 70 Y	specimen collection, 32-33	nonpathologic causes of, 45t	urinalysis and, 262, 263
suprapubic aspiration of, 36 volume, 31–32 Urinometer, 46, 46f Urisys 1100 system, 260t, 261 Urine clarity, 45t normal, 44 turbidity, nonpathologic, 44–45 Urobilin, 69 Urobilinogen, 54, 68, 70 disorders of the phenylalanine-tyrosine Urinometer, 46, 46f Urisys 1100 system, 260t, 261 Urisys 2400 system, 260t, 261–262 Vanthochromatic supernatant, 180 Xanthochromia, 179 Y	specimen handling, 33-34	pathologic, 45	World Health Organization (WHO), 203-204,
volume, 31–32 Urisys 1100 system, 260t, 261 Urine clarity, 45t Urisys 1800 system, 260t, 261 Normal, 44 Urisys 2400 system, 260t, 261–262 Uribidity, nonpathologic, 44–45 Urobilin, 69 Urobilinogen, 54, 68, 70 Urobilinogen, 54, 68, 70 Urobilinogen, 54, 68 of in urine, 70 Y Urisys 2400 system, 260t, 261–262 Xanthochromatic supernatant, 180 Xanthochromia, 179 Y	specimen types, 34-38, 35t	pathologic causes of, 45t	207
Urine clarity, 45t normal, 44 turbidity, nonpathologic, 44–45 Urobilin, 69 Urobilinogen, 54, 68, 70 disorders of the phenylalanine-tyrosine Urisys 1800 system, 260t, 261 Urobisys 2400 system, 260t, 261–262 Vanthochromatic supernatant, 180 Xanthochromia, 179 Vanthochromia, 179 Y	suprapubic aspiration of, 36	Urinometer, 46, 46f	Wright's stain, 95, 182
normal, 44 Urisys 2400 system, 260t, 261–262 Xanthochromatic supernatant, 180 turbidity, nonpathologic, 44–45 Urobilin, 69 Xanthochromia, 179 Urine screening Urobilinogen, 54, 68, 70 disorders of the phenylalanine-tyrosine clinical significance of in urine, 70 Y	,		
turbidity, nonpathologic, 44–45 Urobilin, 69 Urine screening Urobilinogen, 54, 68, 70 disorders of the phenylalanine-tyrosine Urobilinogen, 54, 68, 70 Clinical significance of in urine, 70 Y Xanthochromia, 179 Y	Urine clarity, 45t	Urisys 1800 system, 260t, 261	X
Urine screening Urobilinogen, 54, 68, 70 disorders of the phenylalanine-tyrosine Urobilinogen, 54, 68, 70 Clinical significance of in urine, 70 Y	,		Xanthochromatic supernatant, 180
disorders of the phenylalanine-tyrosine clinical significance of in urine, 70			Xanthochromia, 179
pathway, 164 reaction interference in urine, 70 Yeast, 93f, 100, 101f	1 , ,		
	pathway, 164	reaction interference in urine, 70	Yeast, 93f, 100, 101f